Original article:

Helicobacter Pylori is associated with decrease serum level of the thyroid hormonal in healthy elderly population

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Abstract

Background: *Helicobacter pylori* infection is the most prevalence infectious disease as it affects more than half of the world population and causes chronic cellular inflammatory response in the gastric mucosa. *Helicobacter pylori* infection has been epidemiologically proven to be linked to extra-digestive conditions and disease. It has been speculated that *H.pylori* infection may be responsible for various endocrine disorders. The thyroid may be one of the targets of *Helicobacter pylori* chronic inflammation. Here we sought too investigate whether *H.pylori* infections were associated with decrease level of the thyroid hormonal. ***Methods:*** This study involved elderly aged 50-90 years who had visited a health promotion center for elderly. A total 101 euthyroid subjects were been enrolled in this cross-sectional study. Diagnosed of *Helicobacter.pylori infections* by ELISA of Ig G antibodies of *Helicobacter pylori.* We examine serum T3 level and serum TSH level by ELEXIS. For statistical method we use Pearson bivariat analysis to determine the association of two variable,and linier regression to determine which variable is more influented by *Helicobacter pylori*. ***Results:*** Fourty-two (41,6 %) subjects had been diagnosed with *H.pylori infections*. Pearson bivariat analysis showed that *Helicobacter pylori* infection was significantly associated with decreased serum T3 level (correlations coefficient r = -0,66 ,p< 0,001). The prevalence of *Helicobacter pylori* infection showed a increasing trend as serum TSH level decreased (correlations coefficient r = -0,53, p < 0,001). Linier regression analysis showed that*Helicobacter pylori* infection was significantly associated with the risk of decreased thyroid hormonal fuction (B = -0,272. R^2 = 0,676. P < 0.001). ***Conclusion:*** Our results suggested that *H.pylori* infections were significantly associated with the decreased serum level of T3 and TSH serum level in the healthy elderly population, whose thyroid functions were in the reference range.

Keyword: *Heliccobacter pylori:* serum T3 level: serum TSH level*:* healthy elderly population

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Introduction

Helicobacter pylori (H pylori) is a pathogenic bacterium, gram-negative, spiral-shaped that colonizes specifically at the gastric epithelium. [1,2]

The prevalence of H pylori infection was 36-41.6 % in Indonesia and more over 50 % of all the world population .[2,3] *H pylori*can causes some clinical manifestation such as chronic gastritis, peptic ulcer disease and gastric malignancies. [4,5] The infection induces infiltration of polymorphonuclear that if nor cleared effectively, than replaced gradually by an immunologically-mediated, chronic, and latter induce pro-inflammatory cytokines production by local or systemic,[1,2-6]as aresult, gastric epthelium is not the only one target of *H pylori* pathologic manifestationbut also affected to some extra-digestive conditions, including endocrine disorders, including autoimmune thyroid disorders (ATD) such

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as autoimmune atrophic thyroiditis and Hashimoto's thyroiditis, or thyroid mucosal associated lymphocyte tissue (MALT) lymphoma that has been epidemiologically proven,. [1,7,8] Even there have been controversial report about the link between *H pylori* infection and thyroid disorders, but some studies have been sucsesfully reported an increased prevalence of *H pylori* in people with ATD and relationship between *H pylori* infection and the presence of high titers of thyroid auto-antibodies, such as anti-thyroglobulin (anti-Tg) and anti-thyroperoxidase (anti-TPO) antibodies resulting in abnormalities of gastric secrtory fuction.[1]

Other studies have been demonstrated that some bacteria and viruses are able to mimic the antigenic profile of the thyroid cell membrane, thereby playing an important role in the pathogenesis of autoimmune diseases. Therefore, the thyroid may be the target by autontibodies after *H pylori* infection.[1, 7,8]

Chronic inflammation that caused by *H pylori* can induces oxidative stress that has been shown to be associated with both hyperthyroidism and hypothyroidism.[8,9] however the mechanisms of oxidative stress generated in to hypothyroidism is low availability of antioxidants.[9',10]

Therefore, we conducted a cross-sectional study to investigate whether correlation between *H. pylori* infection and T3 serum level also thyroid-stimulating hormone serum level.

Material and Methods

Study design and subjects

This study was performed in elderly aged 50-90 years who had visited the health promotion center for elderly at Pos Lansia Nusukan, Solo, Indonesia, from Oktober to November 2016, and we take 101 subjects were enrolled (29 men and 72 women, with mean age 73.68 ± 13.00 years) with healthy condition by laboratory and phisical check up who voluntarily participated as subjects in this study. All procedures were approved by the Ethics Committee of Sebelas Maret University of Faculty of Medicine. Each method and the potential risks were explained to the participants in detail, and all subjects gave written informed consent before the study.

Laboratory assessments

All subjects had required to fast overnight prior to laboratory assessments in the morning. Peripheral venous blood samples were collected and used for the analysis of H pylori antibodies titer, T3 serum level and TSH serum level. Serum TSH level and Total T3 had assessed with ELEXIS (Roche). The diagnosis of *H pylori* were determined according to Ig G againts *H pylori* that had assessed with ELISA by PLATOS R496(AMP Diagnostics). Positive H pylori infection if the Ig G againts H pylori more than 2.00 U/mL

Statistical analysis

Statistical analysis was used with SPSS 17.0 statistical package(SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test had used to assess whether continuous data were normally distributed. Continuous variables has been presented the mean, median, standard deviation, minimum value, and maximum value. Categorical variable has been presented as the frequency and percent. Pearson Correlationtest had used to determine the association between H pylori infection and T3 and TSH serum level. The chi-square (x^2) test had used to determine theassociation of categorical variable. Regression linier analysis had used to evaluate the risk factors for decrease T3 and TSH serum level. (backward:Wald; cutoff for entry : 0.05, for removal 0.10). If the differences with P-values less than 0.05 than wewere considered statistically significant.

Results

Subjects characteristics

A total 101 subjects which were elderly population who had visited health promotion center for elderly people called Pos Lansia , enrolled in this study. They were 29 men and 72 women with mean age

Tabel 2. Characteristics categorical variable

Variable	frequency	Percent
Gender		
Male	29	28.7
Female	72	71.3
H pylori		
Positive	42	41.6
Negative	59	58.4
H pylori positive		
Male	13	12.9
Female	29	28.7

73.68± 13.00 years. Of the 101 subjects, 42 (41.6%) were diagnosed with *H pylori* infections. Characteristics subjects according to continuous variable are illustrated in Table. 1.

Tabel 1. Characteristics continuous variable

Variable	n	Mean	Median	SD	Max value	Min value
Age (years)	101	73.68	74.00	6.50	87	60
Ig G H.pylori	101	1.97	1.96	0.18	2.42	1.50
T3 (ng/ml)	101	0.79	0.84	0.34	1.46	0.12
Thyroid-stimulating hormone (uIU/L)	101	1.18	1.12	0.65	2.76	0.11
Age *H pylori* positive	42	75.00	74.50	6.24	87	62
Age *H pylori* negative	59	72.74	72	6.58	85	60

The age average of subjects that positive *H pylori* infection group was older than those in the negative *H pylori* infection group. Characteristics subjects according to categorical variable are illustrated in Table. 2.

Associations between *H pylori* infection, T3 and Thyroid-stimulating hormone

As shown in Table 3, Pearson corelation r between Ig G Hpylori and T3 is – 0.63 (p<0.001). which mean there are a strong , reverse correlation between H pylori and T3 serum level . Higher level of Ig G H pylori causes lower level of T3 serum. Coefficient determinnt R^2 is 39.69 %, that mean *H pylori* infection as a caused lower serum level T3 about 39.69%, the rest of 60.31% caused by other variable. Pearson correlation r between Ig G *H pylori* and TSH serum level is -0.53 (p<0.001) which mean there are reverse correlation between H pylori and Thyroid-stimulating hormone. Higher level of Ig G H pylori causes lower level of TSH serum level. Coefficient determinant R^2 are 28.09 %. It mean *H pylori* infections can causes lower serum lserum level TSH only 28.09%, but the rest of it about 71.91 % caused by other variable.

Associations between Age and H pylori infection, T3 and TSH

Coefficient correlation r is to showed for association between age and *H pylori* is 0.25(p=0.01), which is weak positive association. Between T3 serum level and age variable have coefficient correlation r = -0.47(p<0.001). this correlation are negative that mean more older age, more decrease T3 serum level. Age have reverse weak association with TSH serum level, r= -0.199 (p<0.046). As illustrated in Table 4.

Tabel 4Association between Age, H pylori infection, T3 and TSH serum level

Variable	n	Mean	Median	SD	Pearson Coefficicent Corelation	p
Age	101	73.68	74	6.50	0.25	0.01
Ig G H pylori	101	1.98	1.96	0.18		
Age	101	73.68	74	6.50	-0.47	< 0.001
T3	101	0.79	0.84	0.34		
Age	101	73.68	74	6.50	-0.199	0.046
Thyroid-stimulating hormone	101	1.17	1.12	0.65		

Association between T3 and Thyroid-stimulating hormone

As shown in Table 5, Pearson Coeficient Correlation between T3 and TSH, r is 0.511 (p< 0.001). Serum T3 level have significantly stong relationship with TSH.

Association between T3 , TSH serum level and the prevalence of *H pylori* infection

In order to investigate the association between T3, TSH serum level and the prevalence of H pylori infection, all subjects were classified into three group according to their T3 and TSH levels.

Risk factor analysis for decrase T3 level

Linier regression analysis was performed to evaluate the most strong variable that associated with decrease level T3 serum. The three variables included age, H pylori infection and TSH levels. The results showed that H pylori infection is the most variable that associated to decrease T3 serum level, which is higher titer antibodies of H pylori will cause lower T3 serum level. Variable TSH levels have positive association with T3 serum level, so more lower TSH serum level affected to

Table 3 Assocoatin between H pylori infection and serum level of T3 and Thyroid-Stimulating hormone

Variable	n	Mean	Median	SD	Pearson Coefficient Correlation	p
Ig G H pylori	101	1.98	1.96	0.18	-0.63	<0.001
T3	101	0.79	0.84	0.34		
Ig G H pylori	101	1.98	1.96	0.18	-0.63	<0.001
Thyroid-Stimulating hormone	101	1.17	1.12	0.65		

Tabel 5. Association between T3 serum level and Thyroid-stimulating hormone

Variable	n	Mean	Median	SD	Pearson Coefficient correlation	p
T3	101	0.79	0.84	0.34	0.511	<0.001
Thyroid-stimulating hormone	101	1.17	1.12	0.65		

T3 seum level become more low .Variable age was significantly associated with the risk to decrease T3 serum levels. (Table 6, $p < 0.05$)

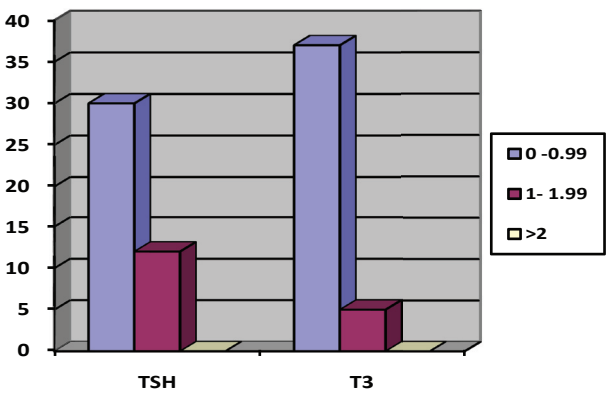
Tabel 6. Analysis Linier Regression for risk factor to decrease serum level of T3

Variabel	B	Std. Error	Beta	t	R square	p
Age	-0.16	0.004	-0.312	-4.333	0.533	<0.001
Thyroid-stimulating hormone	0.111	0.042	0.216	2.637		0.010
H pylori	-0.818	0.155	-0.439	-5.275		<0.001

Association between T3, TSH levels and the prevalence of H pylori infection

As illustrated in Figure 1, for TSH level serum were classified into, group 1 : TSH 0 – 0.99 mIU/L ; group 2 : TSH 1- 1.99 mIU/L; group 3: TSH > 2 mIU/L. For T3, were classifed into, group 1: T3 0- 0.99 pmol/L; group 2 : T3 1 – 1.99 pmol/L; group 3 : T3 > 2 pmol/L.

Figure 1 showed that the prevalence of H pylori infection is more frequently in lower level of TSH serum compared to those high level of TSH serum level. Also in lower level of T3 serum, the prevalence H pylori infection is more frequently compared to those high level of T3 serum. It seems condition lower level of T3 level is most likely tobe related to H pylori infection.



Discussion

Helicobacter pylori is bacterium that cause the most prevalence infection in the world, almost 50% population.[11,12]. It cause dyspepsia, acute gastritis, chronic gastritis, peptic ulcer, MALT-lymphoma and gastric adenocarcinoma.[13, 14, 15] The most virulent strain of *H.pylori* that identified by the presence of cytotoxin-associated gene A (CagA) cause peptic ulcer and gastric carcinoma.[16, 17, 18, 19] Individual with the CagA-positive strain of *H.pylori* infection have an increased risk of peptic ulcer and gastric cancer.[16 18, 20, 19]. The major virulence factor of *H. pylori* infection that affected to the pathological and clinical feature is due to the activity of the cytotoxin-associated gene A (CagA) that cause stimulation of inflammatory responses.[18, 21] Pro-inflammatory cytokines and reactive oxygen species (ROS) that has produced by gastric epithelial cells were contribute to chronic inflammation.[22] Chronic infectious agent such as*H.pylori* to be indicated in the mechanism to the relationship with autoimmune disease has long been suggested and recently has been attention increasingly.[7,21] However, there were no sufficiently data regarding the relathionship between *H.pylori* infection and thyroid autoimmunity. Still need much more research to get more data to proof that in deed there is the link between H pylori infection and thyroid disease.Epidemiological dan serological study have suggested the possibility of a relationship between *Helicobacter pylori* infection and autoimmune thyroid disease such as Graves disease.[7] Because our study was based on data from health check-ups, antibodies of thyroid hormone status was not investigated.

Our data suggested that *H.pylori* infection was significantly linked with the decrease T3 and TSH serum level in elderly population with euthyroid. The prevalence of H. pylori infection was significantly more high in subjects with lower T3 serum level rather than to those without infection. As the same, the prevalence of *H. pylori* infection was significantly more high in subjects with lowerTSH serum level compared to the those without infection. Linier

regression analysis showed that *H.pylori* might be the causal agent for decrease T3 and TSH serum level.H. pylori infection significantly caused to the thyroid hormon disorders. It might be explained by molecular mimicry that infectious agents may lead to thyroid autoimmunity by a variety of mechanisms, such as inducing modification of self-antigens, mimicry of self-molecules, activation of polyclonal T cells, alteration of the idiotype network, formation of immune complexes, and induction of major histocompatibility complex molecules on thyroid epithelial cells.[23, 1] Z.Shen et al. assesed the relathionship between H.pylori infection and the presence of thyroid nodule in 988 patient with euthyroid and found that FT4 serum level were more low in the patients with H pylori infection rather than to those without infection and the prevalence of thyroid nodule was more high in patients with *H.pylori* infection compared to those without infection (odds ratio : 1.390 ; 95% confidence interval 1.059-1.824; p = 0.018).[7] The results of their study were same as our study, but the mechanism was still unclear.

Our study had several limitations. First, we defined *H.pylori* infection based on a serologic test, which detectsboth past and current infections. Second, we did not test for the antibodies of thyroid hormone. Third, we did not test for the presence of CagA because our study was based on health check-up data. However, H.pylori IgG test is highly specific and no cross-reactivity to *Campylobacter coli* microorganism. We excluded subjects with history of thyroid disease.

In conclusion, our findings indicate that H.pylori infection may be associated with decrease T3 and TSH serum level. Further research are more needed to find out deeply understanding about the role of H.pylori infection to thyroid hormone abnormalities..

Key Message

1. H pylori infection have been associted in the pathogenesis of decrease T3 and TSH serum level.
2. T3 serum levels were more low in patient with H pylori infection and TSH serum level was more high in patients with H.pylori infection compared to those without infection.
3. The prevalence of H.pylori infection is highly frequent in subjects with lower level T3 and TSH serum.
4. H. pylori may be associated with decrease level T3 and TSH serum.

Conflict of interest

There no conflict of interest that relevant to this article was reported.

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