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Study serum level of Human leukocyte antigen among patient with Helicobacter pylori Infection

A research submitted to Hilla University College to obtain a bachelor's degree in the Department of pathological analyzes

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(بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ)

﴿ قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا
إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ ﴾

سورة البقرة

(الآية 32)

Supervisor Certification

I certify that this project entitled **(Study serum level of Human leukocyte antigen among patient with Helicobacter pylori Infection)** has been prepared by the students **(Ahmed Ghanem Hasan, Ahmad Karim Hasan, Banan Saedon Muhammad, Zahraa Ahmad Rashid ,Fateima Muhammad Assi, Olaa Muhammad Abd ,Zainab Naeem Fadel)**
under my supervision, in partial fulfilament of the requirement for the degree of bachelor's in medical laboratory technique.

Assist. Lec.
Noor Firas

/ / 2024

According to the recommendations, I nominate this project to be forwarded to discussion.

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/ / 2024

Dedication

I dedicated this study Report

To the purest heart to my role model, and my ideal in life; He is the one who taught me how to live with dignity and loftiness… My dear father

To the heaven of God on earth, to the bridge that ascends me to heaven, to my ideal… My mother

To the eyes and heartbeat You were and still my support, lean and fame in all stages of life. ...to … My brother & sisters

To anyone who helped me and was by my side throughout the study period, to everyone who was happy for my joy and prayed for me with all his heart,

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In the beginning I wish to express my sincere gratitude to Allah for helping me complete my work during very difficult and critical times .

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Abstract

The Gram-negative, spiral-shaped *Helicobacter pylori* microorganism. It mostly affects the stomach's pylorus and leads to chronic gastric infection. It is. The exact method of *H.pylori* infection and transmission through the fecal-oral and oral-oral routes through water or food is yet unknown. Major Histocompatibility Complexes (MHC) include genes called human leukocyte antigens (HLA) that help code for proteins that distinguish between self and non-self. They are important for immunity.

This study aimed to measure the serum level of each the the Human leukocytes antigen -C and the Human leukocytes antigen- DRB1 among patients with *H. pylori* and compared it with Healthy controls. This Case -Control study included 80 blood specimens from addmited patients to the Gastroenterology and Hepatology center of merjan medical city hospital in Babylon province and (50)Healthy controls.

The results of this study revealed that the serum levels of (HLA-C,HLA-DRB1) were measured in patient groups when compared with healthy groups, it was shown the serum levels of HLA-C, It demonstrated asignificant difference (P ≤o.o5) in the serum levels. Patient having a level of 12.89 ng/ml, and controls 6.09 ng/ml, (P ≤0.000). While HLA-DRB1 it was shown the serum levels of HLA-DRB1, in .paitent having a serum level of 1905 ng/ml, and healthy controls 2849 ng/ml, (P ≤0.0003)

The present study provides evidence thát HLA-C It is one of the factors that contribute to the development infections in stomach ulcer patients. it may be a risk factor.while HLA-DRB1 has a preventive effect against bacterial infection.

contents

1-Introduction :

The discovery of *H. pylori*, by Warren and Marshall, was preceded by nearly a hundred year of inconspicuous publications relating to spiral bacteria, achlorhydria, gastritis, gastric urease, and antimicrobial therapy for ulcers (Marshall,1988). Investigation of gastric bacteria properly began in the latter half of the 19th century when microscope resolution had sufficiently advanced (Kidd and Modlin ,1998).

Bottcher and Letulle firstly hypothesized that bacteria caused ulcer disease in 1875, after they discovered bacteria in the floor and margins of gastric ulcers. but they were unable to cultivate them and perhaps because of this they did not present their discovery to the world In 2018, Marshall drank the bacterial suspension himself to prove himself that bacteria are the cause of stomach ulcers and gastritis. After a few days he struck nausea and a foul smell from his mouth, then vomiting appeared. The endoscopy results on the eighth day of the trial were clear - advanced enteritis (Fedorowicz et al., 2020).

In 1889 Walery Jaworski described spiral organisms (*Vibrio rugula*) in gastric washings. He suggested that these organisms might be implicated in causation of gastric disease. Similar spiral organisms were found in stomach of humans and other species by several scientists between then and the 20th century. For instance, in 1893 Bizzozero noted spirochetes in the gastric mucosa of dogs, which were named *H. bizzozeroni*. Kasai and Kobayashi in 1920 isolated spirochetes in cats and transmitted them to rabbits to produce ulcers (Kidd and Modlin ,1998)

Warren in 1979 identified *Campylobacter pylori* as the putative causative agent of human gastritis (Kidd and Modlin ,1998 ; Marshall and Warren,1984) Culture of the organism (*H. pylori*) was elusive until 1982 when it was obtained by Barry Marshall (Marshall,1988). Earlier attempts to culture the organism proved abortive because incubation was usually limited to 48 hours. Success at culture was incidental, as one of them spanned a holiday period and hence, lasted for 5 days, thereby yielding a growth. History was then made in April 1982 at the Royal Perth Hospital in Australia where *H. pylori* was cultured. Examination of the plate showed a pure growth of 1mm transparent colonies. Gram stains of the colonies showed slightly curved organisms and not spiral as in the smear of the specimen, which made Marshall to doubt whether it was the organism in question that was grown. Armstrong and Wee produced electron micrograph scans from the culture obtained, which showed that the bacterium was a spiral organism with five flagella.

Further studies on the organism and its RNA sequence in ribosomes helped correct the earlier misconception that the organism belonged to the *Campylobacter* family (initially called *Campylobacter pyloridis*). At the 5th International *Campylobacter* workshop in Mexico in February, 1989 the *Campylobacter* taxonomy committee agreed that *H. pylori* should no longer be included in the *Campylobacter* group. There was initial difficulty in nomenclature before Steward Goodwin who was head of the Microbiology Department at Royal Perth Hospital at that time reportedly suggested *Helicobacter*, and this was published in 1989.

The World Health Organization classified *H. pylori* as a class 1 (definite) carcinogen implicated in the etiopathogenesis of gastric malignancies in 1994 (Houghton and Wang ,2005) . Parsonnet *et al* also describe an association between

H. pylori and gastric lymphomas(Parsonnet *et al*.,1994). completed sequencing of the entire 1,667,867 base pairs of the *H. pylori* genome in 1997 (Tomb *et al*.,1997) And in 2005 Warren and Marshall were awarded the Nobel Prize in Physiology or Medicine for their work on *H. pylori* and PUD(The Nobel Prize in Physiology or Medicine 2005).

The Aim of study

1-specimens collection(blood and stool)

2-Diagnosis of bacteria through serological test

3-detection serum levels of HLA-C and HLA-DRB1 by ELISA

2-Classification of *H. pylori*

Taxonomy aims to provide a meaningful biological framework upon which a wide range of other sciences is built. However, the inaccurate delineation of taxa serves to confound workers in a variety of disciplines (On, 2001).

Cultivation of new stomach bacteria in 1982 marked a new turning point in our understanding of the environment and diseases of the digestive system. Description Marshall and Warren, spiral or curved bacilli in histological sections of numerous samples of the human gastric mucosa. A number of these samples gave culture positivity to the Gram-negative microaerophilic bacteria. The second international workshop held in Brussels, Belgium, in September 1983 on *Campylobacter* infection gave great importance to its correct classification. The *campylobacter* organism is similar in many aspects, including the curved morphology, growth on rich media failure to fermentation of glucose, sensitivity to metronidazole, and G + C content of 34%. Therefore it was first referred to as

Campylobacter pylori (a doorman, or Greek, or gatekeeper, or a person looking in both directions) and was verified as *Campylobacter* in 1985 . The specific trait was revised to *Campylobacter pylori* in 1987 to correspond with the correct Latin additive for the name pylorus (Solnick and Vandamme, 2001)

3-General Characteristics and Growth Requirement of *H. pylori*

*H*elicobacter pylori (*H. pylori*) is a gram-negative, microaerophilic bacterium that is adapted to survive in the acidic environment of the stomach. Here are some general characteristics and growth requirements of *H. pylori* Morphology is a curved or spiral-shaped bacterium that is approximately 3-4 micrometers long and 0.5 micrometers wide

Motility: *H. pylori* is motile, and it uses several flagella to move through the mucus layer of the stomach. (Qin *et al.*, 2017) .

Growth requirements H. pylori is a fastidious bacterium and has specific growth requirements. It grows best at a pH of 7.0-7.4 and at temperatures between 37-42°C. *H. pylori* requires a microaerophilic environment with low oxygen tension (5-10%) and high carbon dioxide levels (5-10%) for optimal growth. It also requires specific nutrients, such as iron and amino acids, for growth. (Al-Thahab and Al-Awsi, 2018)

Culture H. pylori can be cultured using specialized media, such as Columbia agar supplemented with 5-10% sheep blood and selective antibiotics, such as vancomycin and trimethoprim. (Scott *et al.,2002*

Genome *H. pylori* has a relatively small genome, with approximately 1,500 genes. It has a high degree of genetic variability, with several subtypes or strains that vary in their genetic makeup and virulence factors

Overall, *H. pylori* is a fastidious bacterium that is adapted to survive in the acidic environment of the stomach. Its specific growth requirements and genetic variability may have implications for the development of strategies to prevent or treat *H. pylori*-related diseases. (Lee *et al.*, 2017)

4-Transmission :

H. pylori is primarily transmitted through person-to-person contact, particularly in childhood. The exact mode of transmission is not fully understood, but it is thought to occur through oral-oral or fecal-oral routes.

In areas with high H. pylori prevalence, the bacteria is often acquired during childhood, typically before the age of 10. The exact mechanisms of transmission can vary depending on cultural and environmental factors, but it is thought to involve close contact with family members or caretakers who are infected with *H. pylori* (Aziz, Khalifa and Sharaf, 2015) .

The bacteria can be transmitted through oral-oral contact, such as kissing or sharing utensils, or through fecal-oral contact, such as consuming contaminated food or water. In addition, *H. pylori* can survive in the environment for several days, and contaminated surfaces or objects may also contribute to transmission(Bürgers *et al.*, 2008)

Individuals who live in crowded conditions, have poor sanitation, or are exposed to contaminated food or water are at higher risk of *H. pylori* infection. In addition, certain cultural or dietary practices may also increase the risk of transmission(Gebara *et al.*, 2006).

Overall, the primary mode of transmission of *H. pylori* is person-to-person contact, particularly in childhood. Understanding the mechanisms of transmission

and identifying individuals at risk of infection may be important for developing strategies to prevent or reduce *H. pylori* transmission. (Stefano *et al.*, 2018).

5-Human Leukocyte Antigens (HLA)

Human leukocyte antigen (HLA) molecules play a critical role in the adaptive immune response to *H. pylori* infection. HLA molecules are cell surface proteins that present antigenic peptides to T cells, allowing them to recognize and respond to specific pathogens.

HLA molecules are highly polymorphic, meaning that there are many different variants of HLA genes in the human population. Different HLA variants can present different antigenic peptides, and the ability of an individual's HLA molecules to present *H. pylori* antigens can affect their susceptibility to infection and the outcome of the immune response.

In addition to influencing disease susceptibility, HLA molecules can also affect the immune response to H. pylori. Studies have shown that HLA class II molecules, such as HLA-DR and HLA-DQ, are important for the activation of CD4+ T cells, which play a critical role in the adaptive immune response to H. pylori. The specific HLA alleles expressed by an individual's APCs may affect the types of *H. pylori* antigens that are presented to T cells, influencing the specificity and strength of the immune response.

Overall, the polymorphic nature of HLA molecules can have significant effects on the immune response to H. pylori infection and the development of *H. pylori*-related diseases. Understanding the role of HLA in the immune response to *H.*

pylori may be important for developing more effective diagnostic and therapeutic strategies for these diseases.

(Matsueda *et al***.**2014)

6-Diagnosis of *H.pylori*

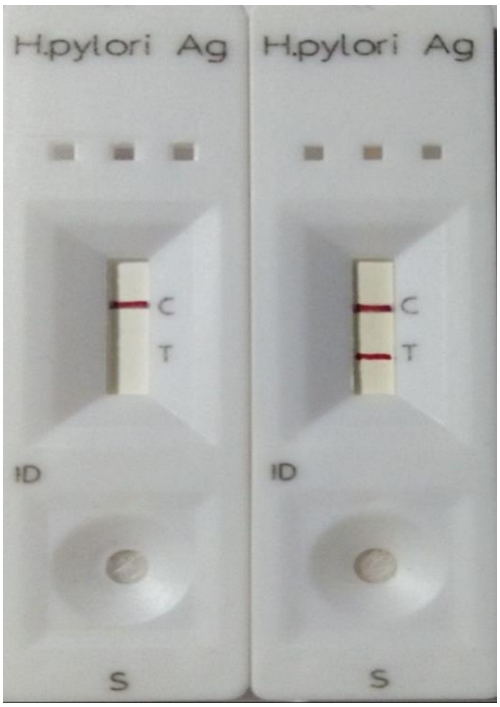
6.1Antibody rapid test

This test was done according to manufacturer company the principle of serum antibody rapid test of *H. pylori* was based on qualitative membrane immunoassay for the detection of *H. pylori* antibodies in whole blood, serum, or plasma . In this test, anti-human is immobilized in the test line region of the test. After sample is added to the sample well of the device, it reacts with *H. pylori* antigen coated particles in the test.

This mixture migrates chromatographically along the length of the test and interacts with the immobilized anti-human . If the specimen contains *H. pylori* antibodies, a colored line will appear in the test line region indicating . The test is used to diagnose the bacteria in blood samples. In the case of infection absence with the bacterium red color appears only on the letter C (control line), while in the case of infection in addition to the package control in red color, a red package will appear on the letter T (result line) as show in Figure (1)

6.2Stool antigen rapid test

The test is used to diagnosis the bacteria in stool samples . small samples of stool specimens collected from three different parts of the stool sample wire transferred to s vial with diluents, vigorously agitated and after two minutes of resting the tuba, dropping sound two to three drops into the round window of the test cassette. Riding was made after 10 minutes of incubation at room temperature, and based on the appearance of colored lines across the central window of the cassette, two lines, C (control) and T (test), indicted positive test, only one line in C indicted negative result. A pale colored line in T was also considered positive as it is shown in Figure (2).



7-Immunological study

7.1 Estimation of serum level HLA-C and HLA-DRB1

ELISA kit was applied to the in vitro quantitative determination of Human HLA-C and HLA-DRB1.

1. **Test principle**

   In the ELISA kit, the Sandwich-ELISA method is used to detect HLA-C and HLA-DRB1, respectively. A Human HLA-DRB1 antibody is pre-coated on a 96-well microtiter plate included in this kit. Samples or standards were mixed with the appropriate antibody in the appropriate wells. A biotinylated detection antibody for HLA-C was then added. To each well, we added an avidin-horseradish peroxidase conjugate (HRP). Washing was performed after the chromatogenic step to remove any remaining free components. Each well was incubated with a different substrate. Only the wells containing HLA-C and HLA-DRB1 contain biotinylated detection

antibodies and Avidin HRP conjugates. The enzyme-substrate reaction turns yellow after the stop solution is added. The optical density (OD) at an excitation wavelength was measured using spectrophotometric technique (450 nm 2 nm). The OD value and Perforin concentration were found to be highly correlated. The concentration of HLA-C and HLA-DRB1. was determined by comparing the OD of the samples to the standard curve

2-Reagent preparation

HLA-C

1- All reagents was brought to room temperature before use.

2- Standard solution preparation: Reconstitute the 120µl of the standard (0.2ng/ml) with 120µl of standard diluent to generate an 80ng/ml standard stock solution. Was allowed the standard to sit for 15 mins with gentle agitation before making dilutions. Prepared duplicate standard points by serially diluting the standard stock solution (80ng/ml) 1:2 with standard diluent to produce 40ng/ml, 20ng/ml, 10ng/ml, and 5ng/ml solutions. Standard diluent serves as the zero standards (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. The dilution of standard solutions

Wash Buffer: Diluted 20ml of washed buffer concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

HLA-DRB1

1- All reagents was brought to room temperature before use.

2- Standard solution preparation: Reconstitute the 120µl of the standard (16.28ng/L) with 120µl of standard diluent to generate a 4800ng/L standard stock solution. Was allowed the standard to sit for 15 mins with gentle agitation before making dilutions. Prepared duplicate standard points by serially diluting the standard stock solution (4800ng/L) 1:2 with standard diluent to produce 2400ng/L, 1200ng/L, 600ng/L, and 300ng/L solutions. Standard diluent serves as the zero standards (0 ng/L). Any remaining solution should be frozen at -20°C and used within one month. The dilution of standard solutions

Wash Buffer: Diluted 20ml of washed buffer concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved

8-Assay procedure

ELISA Assay Procedure

1. Prepared all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.

2. Determined the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. Added 50µl standard to the standard well. Note: Don't added a biotinylated antibody to a standard well because the standard solution contains a biotinylated antibody.

4.Added 40µl sample to sample wells and then add 10µl anti-HLA antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate for 60 minutes at 37°C.

5.Removed the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6.Added 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7.Added 50µl Stop Solution to each well, the blue color will change into yellow immediately.

8.Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution**.**

9-Calculating of Results of ELISA Test

The standard curve was created by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and drawing a best-fit curve through the points on the graph, when specimens have been diluted, the concentration calculated from the standard curve was multiplied by the dilution factor. the OD of the specimen that surpassed the upper limit of the standard curve was tested after appropriate dilution as shown in the figure (3)and (4) .

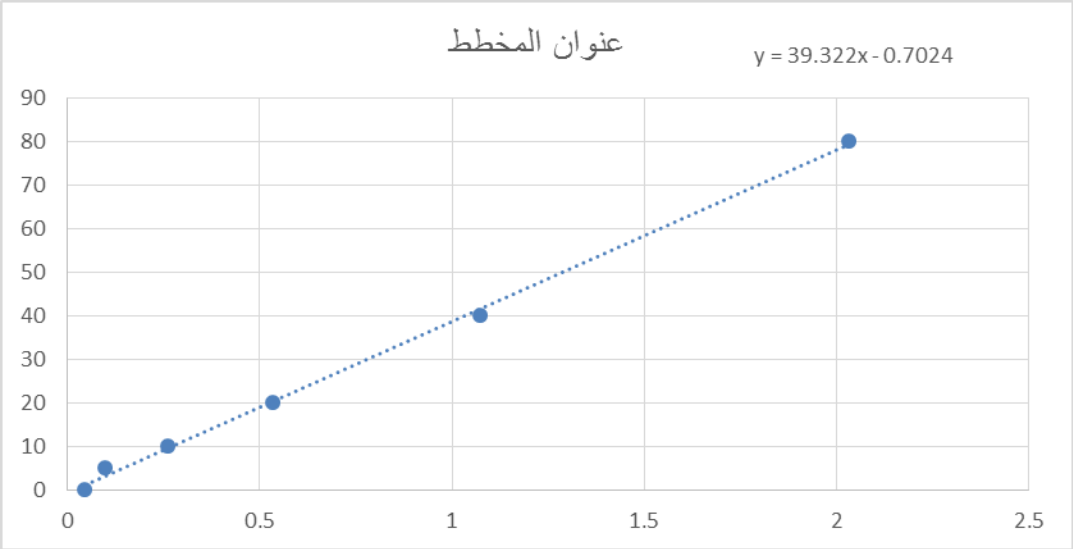


Fig (3): Standard Curve of HLA-C

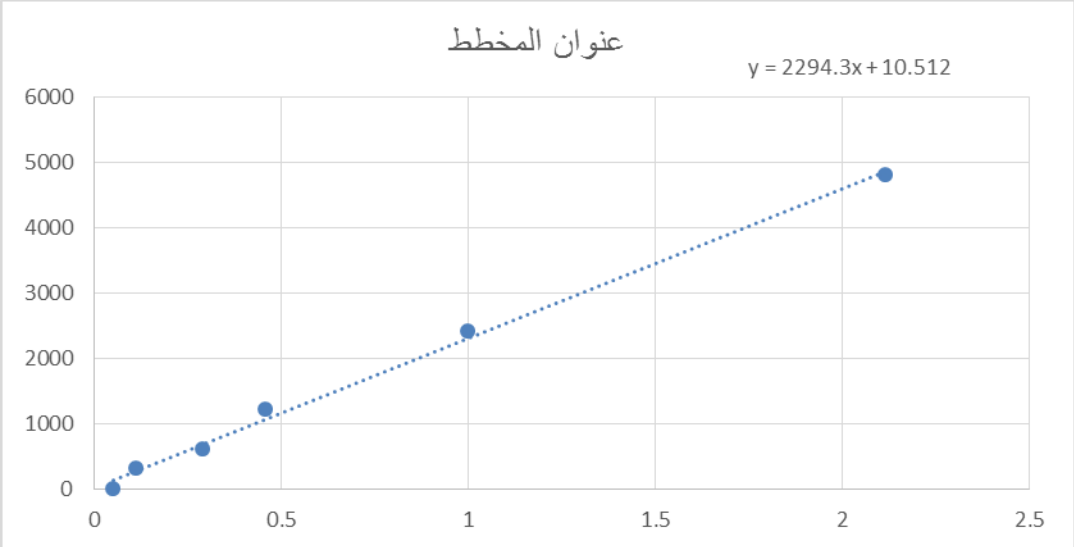


Fig (4): Standard Curve of HLA-DRB1

10-Result

10.1Estimation of HLA-C serum level among *H.pylori* infection.

The results revealed a significant difference (P ≤0.05) in the serum levels of HLA-C in patient when compared with healthy control this study showed an increase in the serum level of HLA-C in patient which was (50.90) ng/L ,compared

with serum levels of healthy control which were (28.77) ng/L, P ≤ 0.000) as shown in Table(1)

Table 1 Estimation of HLA-C serum level(ng/ml)in blood of patients compared with control

Type of patients	No.	HLA-C ng/ml Mean ±SD	P ≤ value
Patients	80	50.90±12.89	0.000
Healthy Controls	50	28.77± 6.09	

10.2 Estimation of HLA-DRB1 serum level among *H.pylori* infection.

The results revealed a significant difference (P ≤0.05) in the serum levels of HLA-DRB1 in patient when compared with healthy control this study showed an decrease in the serum level of HLA-DRB1 in patient which was (1905) ng/L ,compared with serum levels of healthy control which were (2849) ng/L, P ≤ 0.0003) as shown in Table (2)

Table 2 Estimation of HLA-DRB1 serum level(ng/ml)in blood of patients compared with control

Type of patients	No.	HLA-DRB1 ng/L Mean ±SD	P ≤ value
Patients	80	1905±216.1	0.0003
Healthy Controls	50	2849 ± 177	

11-Conclusions

The study reached the following conclusions:

1-This study found an increase in serum level of HLA-C among patients with ***h.pylori*** infection. It may be a risk factor

2-The study It was found decrease in serum level of HLA-DRB1. among patients with ***H. pylori*** infectio .It may be a protective factor

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