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Evaluation of NT-ProBNP and Procalcitonin as Markers from Rodents with Myocardial Toxoplasmosis Using ECL Method: A Pilot Study

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ABSTRACT

Aims *Toxoplasma* parasites that extracted from different rodents are the same in immunologic and morphological characteristics but different in pathogenic characteristics. We found that the serum levels of ProBNP and Procalcitonin markers are high among these rodents. The aim of this study was the assessment of the serum levels of ProBNP and Procalcitonin markers among the rodents with myocardial toxoplasmosis.

Materials & Methods In this study, we collected 286 rodents and extracted 250g of their heart tissues and blood samples to obtain DNA of *T. gondii*. We detected the positive samples, using the nested PCR method. Then, we examined serum levels of Pro BNP and Procalcitonin markers, using Electro Chemo Luminescence method (ECL) for assessment of myocardial toxoplasmosis in this host. Data analysis was also conducted by the statistical analysis method. This study was performed from January to March 2017, based on the prevalence study.

Findings In this study, 68/286 samples of rodents were positive for *GRA6* gene and these positive samples had high levels of Pro BNP and Procalcitonin markers that indicated myocardial toxoplasmosis and acute inflammation among these animals.

Conclusion In this study, we found that the *GRA6* gene was very useful to follow up toxoplasmosis in the rodents of the Golestan province, northeast of Iran. Also, ProBNP and Procalcitonin markers were at high levels in myocardial toxoplasmosis.

Keywords Toxoplasmosis; Rodents; *GRA6* Gene; Pro BNP Marker; Procalcitonin

CITATION LINKS

[1] Production and evaluation of Toxoplasma gondii recombinant surface antigen ... [2] Serologic survey of toxoplasmosis in Seoul and Jeju-do, and a brief review ... [3] Detection of ocular Toxoplasma gondii infection in ... [4] Genetic characterization of viable Toxoplasma gondii isolates ... [5] Toxoplasma gondii abortion storm in sheep on a Texas farm ... [6] Isolation and genetic characterization of Toxoplasma gondii ... [7] Genetic characterisation of Toxoplasma gondii isolates from ... [8] High prevalence of Toxoplasma gondii oocyst shedding ... [9] Genetic characterization of Toxoplasma gondii from ... [10] First isolation and genotyping of Toxoplasma gondii ... [11] Seroprevalence, detection of DNA in blood and milk, and ... [12] Prevalence and risk factors of Toxoplasma gondii ... [13] Seroepidemiology of Toxoplasma gondii infection in drivers ... [14] DNA detection of Toxoplasma gondii with a magnetic ... [15] Prevalence and genetic characterization of ... [16] Detection and genotyping of Toxoplasma gondii ... [17] Cardiovascular disease biomarkers are ... [18] Elevated admission N-terminal pro-brain natriuretic peptide ... [19] The REFER (REFer for EchocaRdiogram) study ... [20] Development of a BNP1-32 immunoassay that does ... [21] Copeptin and NT-proBNP for prediction of all-cause ... [22] NT-proBNP predicts mortality in adults with ... [23] Comparative evaluation of copeptin and ... [24] The relationship of plasma creatinine ... [25] Relation of N-Terminal Pro-B-Type natriuretic peptide ... [26] Plasma N-Terminal probrain natriuretic ... [27] The prognostic value of pro-calcitonin ... [28] Postoperative serum pro-calcitonin and C-reactive ... [29] Elucidation of the sequence of canine ...

Introduction

Pro B-type Natriuretic Peptide (BNP) and procalcitonin markers are important proteins that arise in blood flow of the patients with heart failure disorders and inflammation. *Toxoplasma gondii* is a widespread organism with worldwide distribution, which infects many warm-blooded hosts, including humans and rodents. It is estimated that nearly 30% of the human population in the world have this infection. Because the rodents are the natural reservoir hosts of the *Toxoplasma gondii*, they play an important role in spreading of the disease. Rodents as generally considered as relevant hosts to evaluate local contamination by toxoplasmosis. Comparing the infected and uninfected rodents, the recent studies have suggested that tissues of rodents play the main role in the transmission of the toxoplasmosis to other animals. Toxoplasmosis caused by the protozoan *T. gondii* can lead to encephalitis, retinitis, neural tube defects (NTD), and myocarditis. *T. gondii* infects WBCs primarily and is capable of invading and dividing within a wide variety of nucleated host cells [1-5]. Pro BNP and Procalcitonin markers are also the main proteins that increased in cardiovascular and inflammatory disorders such as myocardial toxoplasmosis. The purpose of this study was the assessment of serum levels of Pro BNP and Procalcitonin markers among the rodents with myocardial toxoplasmosis detected by the nested-polymerase chain reaction (PCR) methods and using *GRA6* gene [6-10].

Materials and Methods

Study area: In the Golestan province, rodents are the main reservoir of toxoplasmosis dissemination. This study was conducted from January to March 2017. We collected 286 heart tissues and blood samples of these rodents in that area to examine the nested-PCR using *GRA6* gene. Then, the Pro BNP and Procalcitonin markers were analyzed in the serums of this host, using Electro Chemo Luminescence method (ECL) by COBAS e411 auto analyzer instrument. This study was based on the prevalence study. Golestan province has very humid, sultry weather, and optimum condition for *T. gondii* growth.

Sampling: We collected 286 rodents from Golestan province to examine the infection status of visceral organs. 250g of the heart tissue of the rodents were removed. These organs were fixed in 95% Ethanol and preserved at 4˚C until DNA extraction. Then, we used nested-PCR method to detect positive samples.

Host typing: The hosts under investigation were *Rattus Rattus*, *Rattus norvegicus*, *Mus musculus*, and *Rombomys opimus.* These rodents of the Golestan province were divided into 4 groups including *Rattus rattus*, *Rattus norvegicus*, *Mus musculus*, and *Rombomys opimus*.

DNA extraction: Genomic DNA was extracted from 3g heart tissues by sodium DNA Extraction Solution (DNG-plus) method from Sinacolon Company and eluted into 50µl DDH2O according to the manufacturer's recommendations.

Nested-PCR analysis for *T. gondii GRA6* gene: Nested-PCR analysis was performed to detect *Toxoplasma GRA6* gene as a diagnostic gene according to Pishgam Company order.

Amplification of the *GRA6* gene was completed under the following conditions: 1 cycle of 5min at 95˚C for initial denaturation followed by 30 cycles of 1min at 95˚C, 1min at 62˚C, and 3min at 74˚C. The best annealing temperature was 62˚C. Amplification was performed using a DNA thermal cycler (Eppendorf; Germany). PCR amplification products were examined in 1.5% agarose gels and confirmed by staining with Safe stain and visualized under Gel Doc using UV.

Then, we evaluated the serums of toxoplasmosis rodents to detect Pro BNP and Procalcitonin markers, using ECL method. The positive control for PCR was Tehran strain of *T. gondii* and for ECL was blood sample of BALB/C mice suffered from cardiac MI.

Statistical analysis: The statistical analysis was done by two-way ANOVA, high low bars, Errors bars and frequency curve with SPSS19 and PRISM software.

Findings

In this study, we found that 68 samples of the rodents were positive in the presence of *GRA6* gene. 38 samples were *R. ratus*, 10 samples were *R. norvegicus*, 10 samples were *M. musculus* and 10 samples were *R. opimus*. These samples were positive in the 344bp band.

Pro BNP and Procalcitonin markers are the main protein and factor that increased in myocardial disorders such as heart failure and acute inflammatory disorders. The normal range of this factor in serum is up to 25pg/ml for Pro BNP and up to 0.5mg/ml for Procalcitonin. The elevated levels of this factor approved cardiovascular diseases. In this study, we detected that positive samples of the infected rodents had high levels of Pro BNP and Procalcitonin markers.

In Diagrams 1-4, we showed that the serum levels of Pro BNP and Procalcitonin markers among the rodents with toxoplasmosis were over 25pg/ml and 0.5mg/ml, respectively. Pro BNP and Procalcitonin markers levels evaluated by autoanalyzer COBAS e411 instrument ECL based. Among the uninfected rodents (Negative for *GRA6* gene) the serum levels of Pro BNP and Procalcitonin markers were in normal range.

These results showed positive samples in 344bp area with *GRA6* gene. Among 131 samples from *R. rattus*, 38 samples were positive, among 45 samples from *R. norvegicus*, 12 samples were positives, among 60 samples from *M. musculus*, 10 samples were positive and, finally, among 50 samples from *R. opimus*, 8 samples were positive. The samples were from rodent's heart tissue (Diagram 5).

In Table 1, we compare the serum levels of Pro BNP and Procalcitonin markers by the two-way ANOVA study. The mean of Pro BNP levels among the infected rodents was 1720pg/ml and the mean of Procalcitonin levels was 6.867mg/ml. These results approved that, Pro BNP and Procalcitonin markers were at high levels among the rodents with myocardial toxoplasmosis.

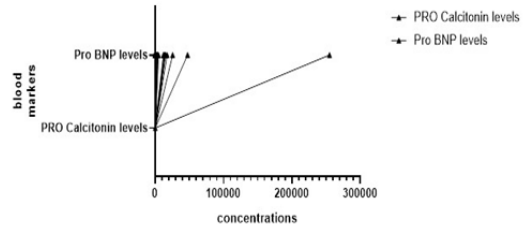

Diagram 1) Comparative study between serum concentration of Pro BNP and Procalcitonin in rodent with toxoplasmosis (linear graph)

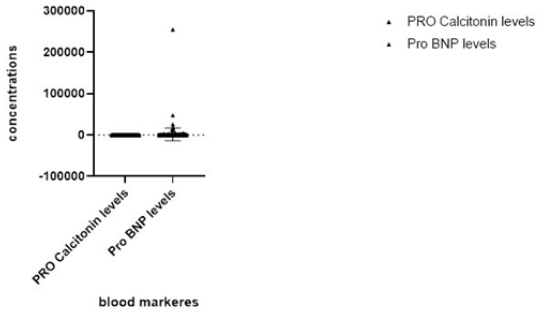

Diagram 2) Comparative study between serum concentration of Pro BNP and Procalcitonin in rodent with toxoplasmosis (high low bars)

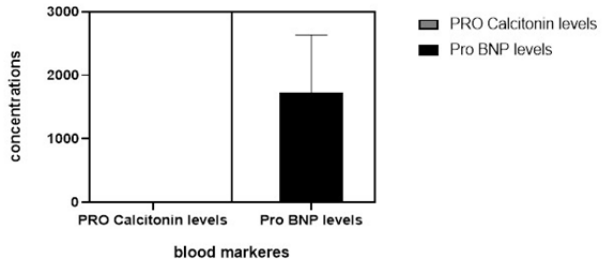

Diagram 3) Comparative study between serum concentration of Pro BNP and Procalcitonin in rodent with toxoplasmosis (high low bars) with 95% confidence interval

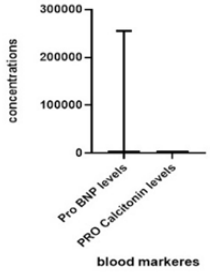

Diagram 4) Comparative study between serum concentration of Pro BNP and Procalcitonin in rodent with toxoplasmosis (Errors bars)

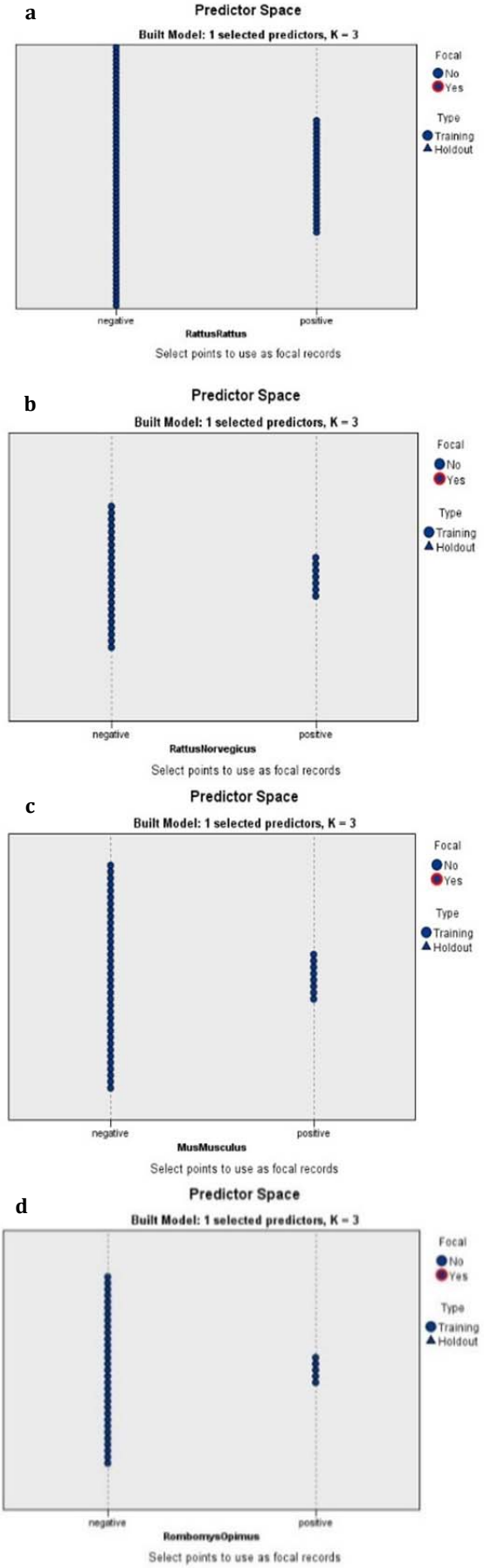

Diagram 5) Comparative study between four groups rodents in the presence of T. gondii *GRA6* gene

Table 1) Two-way ANOVA of serum concentration of Pro BNP and Procalcitonin in rodents with toxoplasmosis

Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Row Factor	49.70	0.4991	ns	No	
Column Factor	0.6082	0.0628	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	34297322722	285	120341483	F (285, 285)=1.000	P=0.4991
Column Factor	419678202	1	419678202	F (1, 285)=3.488	P=0.0628
Residual	34288212760	285	120309518		
Difference between column means					
Mean of Pro BNP levels	1720				
Mean of PRO Calcitonin levels	6.867				
Difference between means	1713				
SE of difference	917/2				

Discussion

The aim of this study was the assessment of serum levels of Pro BNP and Procalcitonin markers among the rodents with myocardial toxoplasmosis detected with the nested-PCR method in the presence of *GRA6* gene. The main reservoir of toxoplasmosis in the northeast of Iran is rodents. Regarding the free-living rodents and many different hosts, as well as their presence in large numbers in rural areas, obtaining information about *T. gondii* circulating pathway among the rodents and other animal population in rural areas is critical for following up the disease [9-11]. In the studied samples, the toxoplasma parasite gene was first detected among the rodents. The specimens were tissues extracted and separated from the rodents in the laboratory. In the first stage, 68 samples were confirmed for Toxoplasma parasite. Then, the rate of inflammatory factors in the blood of the infected rodents was assessed. It was found that in the infected rodents with Toxoplasma parasites, these factors were at a very high level, confirming cardiac toxoplasmosis. These two factors (Pro BNP and Procalcitonin) play a significant role in tracking heart disease as well as blood infections. This surveillance was based on a comparative prevalence study. Golestan province is located in northeast of Iran with a very humid and sultry climate. In Golestan province, the rodents, stray cats and domestic animals such as cattle, are the main reservoir for dissemination of toxoplasmosis. One of the main problems of this study was simultaneously evaluating serum levels of these markers and *GRA6* gene of *T. gondii*. The necessity of this study was the important correlation between the serum levels of Pro BNP and Procalcitonin markers and cardiac toxoplasmosis. Rodents are the main reservoir host for toxoplasmosis to infect cats. Moreover, the uncooked meat of domestic animals such as cattle or sheep can transfer toxoplasmosis to human. Climate characteristic of Golestan province is highly suitable for dissemination of toxoplasmosis. Forty percent prevalence of *T. gondii* antibodies among the cats in Sari, the northeast of Iran, has been detected by Sharif *et al*. Sharif *et al*. studied *T. gondii* antibodies with latex agglutination test on 100 serum samples gathered from stray cats in five areas of Sari province [12-15].

Jamali in Tabriz clarified 36.2% *T. gondii* infection of cats by the dye test that differed with our method. In this study, most positive samples belonged to Golestan villages. In Sari, by contrast, differences in *T. gondii* infection were detected between male and female stray cats. In 2013, Cong detected house sparrows toxoplasmosis in China. Khademvatan detected birds toxoplasmosis in the southwest of Iran and Ortega found pigs toxoplasmosis in Mexico [12-16].

In recent years, no study detected the rodents toxoplasmosis and myocardial markers at the same time in Golestan area. These studies showed an important role of the rodents in the dissemination of toxoplasmosis in the humid area. In the present study, we showed that the *GRA6* gene was a very important marker of the abundance of zoonotic toxoplasmosis, and the heart tissue was very important to find *GRA6* gene in tachyzoites of *Toxoplasma* parasite [16].

Pro BNP and Procalcitonin markers are the main protein and factors that increase in myocardial disorders such as heart failure. The normal range of this factor in serum is up to 25IU/ml for Pro BNP and up to 0.5mg/ml for Procalcitonin. The elevated levels of this factor was revealed in cardiovascular diseases. The purpose of this study was the comparative study between levels of serum Pro BNP and Procalcitonin markers in rodents with myocardial toxoplasmosis. The infected rodents with toxoplasmosis were detected by nested-PCR method and using *GRA6* gene. 23.77% of infected rodents with the myocardial toxoplasmosis were also increase in serum levels of Pro BNP and Procalcitonin markers. Pro BNP and Procalcitonin markers are markers produced in the heart and released when the heart is stretched and is working hard. Many studies have been reported to evaluate of inflammatory factors and heart failure markers to follow up the disease such as cardiac MI and other vasculitis, but no studies have ever been done on the relationship between cardiac toxoplasmosis and

inflammatory factors in the rodent's blood [17-29].

Conclusion

In this study, we concluded that the northeast of Iran was a very important region for following up the toxoplasmosis infection, specifically among the animals such as rodents. Also, Pro BNP and Procalcitonin markers elevated in the serum extracted from rodents with myocardial toxoplasmosis.

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Ethical permissions: The thesis ethics of students, Ehsan Shariat Bahadori with subject Toxoplasma genotyping in wildlife from northeast of Iran and animal analysis was approved by 52/3595 number on 2015 by Ethics committee, provided that it does not lead to animal distress, Tarbiat Modares University.

Conflicts of interests: The authors declared no conflicts of interest.

Authors' Contribution: Shariat Bahadori E. (First author), Introduction author/Methodologist/ Original researcher (20%); Sadraei J. (Second author), Methodologist/Original researcher (50%); Dalimi A. (Third author), Statistical analyst/Discussion author (30%)

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