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Interferon- Gamma- Inducible Guanosine Triphosphate Cyclohydrolase 1 (GTP-CH1) Pathway Is Associated with Frailty in Egyptian Elderly

Magda Mohamad*[1], Somaia Ebeid[2],
Mohamed Shawky Khater[2], Mohamad Alsadany[2]

Abstract

Background: Chronic low-grade inflammation may be a cardinal pathophysiologic feature in the pathogenesis of frailty. Interferon-gamma (INF-γ) is an understudied proinflammatory cytokine in frailty that induces many inflammatory pathways including the guanosine triphosphate cyclohydrolase 1 (GTP-CH1) pathway. Our aim was to evaluate the GTP-CH1 pathway in Egyptian frail elderly subjects.
Methods: INF-γ, neopterin, and nitric oxide (NO) levels were measured in 80 participants.
Results: Both pre-frail and frail subjects had significantly higher levels of INF-γ, neopterin and lower levels of NO than the control group. These biomarkers were associated with the risk of frailty with significant odds ratio.
Conclusions: Elevated INF-γ levels in frail subjects may activate the GTP-CH1 pathway. Elevated neopterin and reduced NO levels correlated with an active GTP-CH1 pathway. The risk of frailty increased with elevated INF-γ and neopterin and decreased with elevated NO levels.

Keywords: Frailty, GTP-CH1 pathway, INF-γ.

Introduction

Frailty, a common geriatric syndrome characterized by multi-system dysregulation, is associated independently with morbidity and mortality due to decreased physiologic reserve and increased vulnerability to adverse health outcomes (1). The prevalence of frailty in community-dwelling elderly adults varies from 4 to 59.1% and increases with increasing age (2). In a recent meta-analysis, inflammatory markers including interleukin (IL) -6 and C-reactive protein (CRP) reported in 31 cross-sectional studies were greater in frail than in robust subjects, suggesting the role of inflammation in frailty onset (3).

Interferon-gamma (INF-γ), a T helper 1 (Th-1) -type proinflammatory cytokine, plays a crucial role in macrophage activation and cytokine production (4). INF-γ production was greater in senescence-accelerated mice than in controls (5). INF-γ induces guanosine triphosphate cyclohydrolase 1 (GTP-CH1), which is the rate-limiting enzyme of tetrahydrobiopterin (BH4) biosynthesis from guanosine triphosphate (GTP). In human monocyte-derived macrophages and dendritic cells the upregulation of GTP-CH1 expression is not accompanied by upregulation of pyruvoyl tetrahydropetrin synthase (PTPS), which leads to subsequent accumulation of dihydrobiopterin (BH2) and its stable metabolite neopterin at the expense of BH4. The latter is a cofactor of many enzymes as nitric oxide synthases (NOS) that convert L-arginine to L-citrulline and nitric oxide (NO) (6). The INF-γ-inducible GTP-CH1 pathway was reported in aging, inflammation, and aging-associated psychiatric disorders (7).

Neopterin is produced by monocytes and macrophages upon stimulation with INF-γ. Elevated

1: Medical Biochemistry Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.
2: Geriatrics and Gerontology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.
***Corresponding authors: Magda Mohamad; Tel: +201006582621; Fax: +201006582621; E-mail: magdaibrahim_bio@yahoo.com.**
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neopterin levels reflect activation of GTP-CH1 by INF-γ, but not by other proinflammatory factors such as IL-1 beta. Interluekin-1 beta activates mainly PTPS and partly GTP-CH1 with an increase in BH4 and decreases in BH2 and neopterin (8).

Our aim in this work was to evaluate the association of the INF-γ-inducible GTP-CH1 pathway in frailty by measuring INF-γ, neopterin, and NO levels in Egyptian elderly.

Materials and methods

Eighty subjects aged 60 years and older were recruited from the outpatient clinic of the Geriatrics Department at Ain Shams University Hospital, Cairo, Egypt. Subjects with active infections, Parkinson's disease, stroke with residual hemiparesis, symptomatic congestive heart failure, malignancy, autoimmune diseases, uncompensated endocrine disorders, rheumatoid arthritis or any other chronic inflammatory conditions, or taking immune modulating drugs including oral steroids were excluded. Those with a significant cognitive deficit (Folstein mini-mental status exam score below 18/30) were also excluded.

This study followed the guidelines of Medical Ethical Committee, Faculty of Medicine, Ain Shams University. Each participant was subjected to the following after taking consent:

1) Frailty was defined using Fried's criteria: unintentional weight loss, exhaustion, muscle weakness, slowness while walking, and low activity levels. Those who met at least three of the five criteria were defined as "frail," while those not meeting any of the criteria were defined as "robust." If the subjects had one or two criteria; they were defined as ''pre-frail' (9). Of our 80 subjects, 26 were defined as robust, 22 as pre-frail, and 32 as frail.

2) Comprehensive geriatric assessments, including screening for dementia using the Arabic version (10) of the mini-mental state examination (MMSE) (11). Subjects were screened for depression using the Arabic version (12) of the geriatric depression scale (GDS-15 item) (13). Functionality was assessed using the activities of daily living (ADL) and instrumental activity of daily living (IADL) scales (14). Mobility was assessed using a timed up and go test (15). Nutritional status was evaluated with the (MNA) mini nutritional assessment (16).

3) Body mass index (BMI) was calculated as the body weight in kilograms divided by height in meters squared (kg/m^2).

Laboratory measures

Peripheral venous blood samples were collected. Serum was isolated by centrifugation and stored at -80 ºC until analysis. Both INF-γ and neopterin were measured using commercially available enzyme-linked immunosorbent assays (ELISA) (Sunlong Biotech Co., Zhejiang, China). Nitric oxide was measured by a previously described method (17).

Statistical analysis

All statistics were analyzed using SPSS for Windows version 19 (SPSS Inc., Chicago. IL, USA). Values were expressed as mean–±SD for parametric data or medians (25^{th}-75^{th} percentile) for non-parametric data. Analysis of variance (ANOVA) or Kruskal-Waills H tests were used to determine significance among the study groups. Spearman's correlation analysis was performed to evaluate the relationship between the measured biomarkers and some of the clinical data. Linear regression analysis was used where neopterin and NO were the dependent variables and INF-γ was the independent variable. Multinomial regression models were implemented to calculate odds ratios (ORs) of the measured biomarkers after covariates adjustment. Receiver operating characteristic (ROC) analysis was used to assess the performance of the measured biomarkers in robust versus frail subjects. Thus, the resulting sensitivity and specificity detected the best cut-off point of each biomarker and their combinations. Positive predictive values (PPVs) and negative predictive values (NPVs) were calculated to assess the accuracy of these markers in frailty prediction. *P* values <0.05 were considered significant.

Results

The characteristics of our participants and laboratory measures are listed in Table 1.

Spearman's rank correlation analysis revealed that INF-γ was positively correlated to neopterin (r = 0.576,

P= 0.001), and negatively correlated to NO (r = - 0.25, *P* =0.03). No significant correlation was detected between neopterin and NO (r = 0.07, *P* = 0.6). The geriatric depression scale (GDS) was positively correlated to INF-γ (r = 0.24, *P* = 0.03) and neopterin (r = 0.37, *P* = 0.001). No significant correlations were detected between the biological markers and ADL, IADL, or MMSE (data not shown).

Table 1. Demographic characteristics and laboratory measures in frailty categories

	Robust (n= 26)	**Pre-frail (n= 22)**	**Frail (n=32)**	***P*-value**
Age (yrs)	64(62-73.3)	65(61-75)	65(61.8-68.3)	0.5
Gender (M/F)	12/14	16/6	14/18	0.082
MMSE	27(25.5-27.3)	25(23-28)	27(24-27)	0.393
GDS	3(1.3-4)	4(3-4)	4(2-4)	0.1
Non-depressed/depressed	22/4	22/0	26/4	0.17
ADL score	6(5-6)	6(0-6)	5(2-6)	**0.001**
IADL score	8(7-8)	8(3-8)	3(1-6.3)	**0.001**
MNA score	12(7.8-13.8)	12(12-13)	12(12-12.3)	0.699
BMI (kg/ m^2)	29.5±5.7	31.8±4.8	25.5±5.3	0.05
INF-γ (pg/ml)				
Median (25th-75th percentile)	0.75(0.55-0.89)	1.35(1-2.8)	1.47(0.98-1.74)	**0.001**
Mean rank	20.1	52.14	49.1	
Neopterin (pmol/ml)				
Median (25th-75th percentile)	89(75.3-92.8)	114(89.6-139.2)	100.7(83.3-152.8)	**0.006**
Mean rank	28.7	44.4	47.4	
Nitric oxide (µmol/L)				
Median (25th-75th percentile)	242.5(143.8-322.5)	77(28.8-131)	102(75-197.2)	**0.001**
Mean rank	59.2	25.1	35.9	

- MMSE; mini mental score examination, GDS; geriatric depression scale, ADL; activity of daily living, IADL; instrumental activity of daily living, MNA; mini-nutritional assessment, BMI; body mass index, INF-γ; interferon gamma.
- Data are expressed as median (25th-75th percentile), mean rank, or numbers.
- *P* values were significant if *P*<0.05.

Table 2. Linear regression analysis of neopterin and nitric oxide (dependent variables) and INF-γ (independent variable)

	Neopterin Unstand. coefficient B (SE)	***P*-value**	**Nitric oxide Unstand. coefficient B (SE)**	***P*-value**
Model I				
Constant	105.8	----	165.7	---
INF-γ	12 (2.3)	**0.001**	- 4.8 (2.5)	**0.06**
Model II				
Constant	11	----	-14	---
INF-γ	**12.58(2.3)**	0.0001	- 3.77 (2.5)	0.14
Age	0.78	0.41	1.4	0.155
Gender	29.7	0.19	56.8	0.02

- Model I: crude model, Model II; after adjustment for age and gender.
- *P* value was significant (*P* < 0.05)

Table 3. The association between the measured biomarkers and the risk of frailty.

		Model I	Model II	Model III	Model IV
INF-γ					
	pre-frail	29.4 (4.8-179.5)*			23.2 (2.5-214.7)*
	Frail	25.6 (4.2-155.4)*			12 (1.5-99.8)*
Neopterin					
	pre-frail		1.016 (1-1.03)*		1.01(0.97-1.05)
	Frail		1.02 (1-1.032)*		1.02(0.99-1.06)
Nitric oxide					
	pre-frail			0.98(0.97-0.99)*	0.97 (0.96-0.99)*
	Frail			0.99(0.98-1)*	0.98 (0.97-0.99)*

- Models I, II, III were multinomial regression models for INF-γ, neopterin, and nitric oxide respectively. Model IV was for all measured biomarkers. The reference category was robust. INF-γ, neopterin, and nitric oxide were the continuous variables.
- Data were represented as odds ratio (95% CI). The odds ratio was calculated after adjustment for age and gender. **P* value was significant (*P*<0.05).

Table 4. The performance and predictive values of the individual biomarkers and their combinations

	AUC (SE)	Sens.	Spec.	PPV	NPV
INF-γ	0.85(0.05)	67.7%	96.3%	95.5%	72.2%
Neopterin	0.72(0.07)	68%	88%	87.5%	70.6%
Nitric oxide	0.81(0.05)	54.8%	96%	94.4%	65%
INF-γ + Neopterin	0.85(0.06)	80.7%	88.9%	89.3%	80%
INF-γ + Nitric oxide	0.91(0.05)	87 %	96.3%	96%	86.7%
Neopterin + Nitric oxide	0.87(0.05)	67.7%	96.3%	95.5%	72.2%
INF-γ +Neopterin+ Nitric oxide	0.92(0.05)	87.1%	96.3%	96.4%	87%

- AUC; area under the curve, SE; standard error, Sens; sensitivity, Spec; Specificity, PPV; positive predictive value, NPV; negative predicative value.

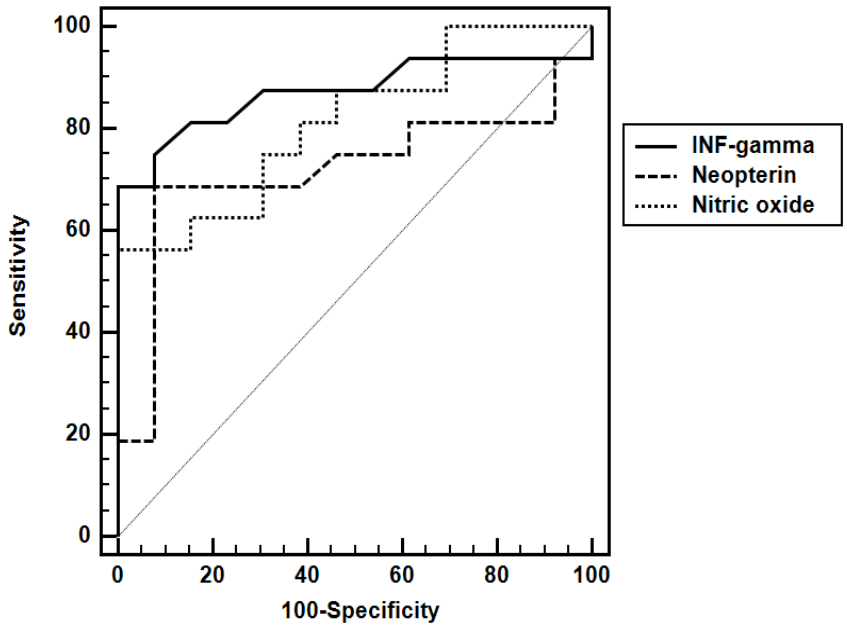

Fig. 1. ROC curve analysis for INF-γ, Neopterin, and Nitric oxide

B-coefficients were calculated to assess the effect of the independent variable INF-γ on the dependent variables neopterin and NO (Table 2).

Odds ratios of INF-γ, neopterin, and NO were calculated in model I, II, and III respectively (Table 3). A one-unit increase either in INF-γ or neopterin increased the pre-frailty and frailty risk while a one-unit increase in NO decreased the frailty risk. All laboratory measures were enrolled in model IV and all biomarkers except neopterin preserved their significant ORs.

Receiver operating characteristic curves were determined for each laboratory measure (Fig. 1). The best cut-off value for each marker was determined to distinguish between frail and robust. The cut-off value was 1.1 pg/ml for INF-γ, 94.7 pmol/ml for neopterin, and 102 µmol/L for NO. The sensitivity, specificity, PPV, and NPV of the biomarkers are shown in Table 4. Furthermore, multiple ROC curves were analyzed for combined biomarkers. The sensitivity was markedly increased when the biomarkers were combined. The best performance and the better PPV and NPV were detected in the combination of INF-γ, neopterin, and NO (Table 4).

Discussion

Aging is characterized by chronic low-grade inflammation. The term inflammaging describes low-grade inflammation in the absence of infection, known as sterile inflammation, in which adaptive immunity declines, and changes in innate immunity could result in mild hyperactivity. This peculiar inflammatory state is detrimental to longevity and has been associated with morbidity and mortality in the elderly (18). The identification of these inflammatory pathways might modulate inflammaging and aid in its treatment. In the aging process a shift towards proinflammatory cytokines, including INF-γ, that induces the GTP-CH1 pathway (19).

Our aim was to evaluate the association of the INF-γ-inducible GTP-CH1 pathway with frailty by measuring INF-γ, neopterin, and NO levels. Greater INF-γ and neopterin and lesser NO levels were observed in the frail and pre-frail in comparison to the robust subjects. Moreover, INF-γ correlated positively with neopterin and negatively with NO. A one-unit increase in INF-γ was associated with a 12-unit increase in neopterin and a 4.8 unit decrease in NO.

As described above, increased neopterin levels reflect INF-γ activity and immune system activation (20); also, neopterin is a marker of GTP-CH1 activity. In addition, increased INF-γ levels indicate that the GTP-CH1 activity is not associated with PTPS activity. Thus, neopterin was produced at the expense of BH4 which is an NOS cofactor. Decreased BH4 synthesis results in decreased NO (7).

In agreement with our findings, increased INF-γ levels were reported in a transgenic frail mouse model (21). Neopterin levels were greater in the pre-frail and frail than in the robust subjects (22) and associated with decreased survival in the elderly (23).

Interestingly, no differences in biomarker concentrations were found between our frail and pre-frail subjects. Our small sample size may be the cause. Thus, large-scale studies are essential to clarify this matter. In our study, the pre-frail and frail risks increased with increased INF-γ or neopterin levels while the risks decrease with increased NO. Also, we observed that these biomarkers may predict frailty, and the best predictive values were obtained if they were combined.

Unfortunately, we were unable to evaluate the association between the GTP-CH1 pathway and depression as the number of subjects with depressive symptoms was small (n=8). However, many studies discussed the potential role of the GTP-CH1 pathway in depression. Depressed patients had decreased NOS activities and low NO levels (24), BH4 deficiencies (25), or elevated neopterin (26). In addition, BH4 deficiency results in NOS uncoupling, with shifting of arginine metabolism away from NO synthesis towards reactive oxygen species (ROS) production (27). Subsequently, ROS activate many inflammatory enzymes involved in depression (28) and can destroy BH4 released from other cells (29). In addition, serotonin production is diminished due to decreased BH4, a tryptophan hydroxylase cofactor (6).

We did not analyse a possible relationship between the measured biomarkers and cognitive function assessed by MMSE, as subjects with cognitive deficits were excluded. However, it was noted that neopterin levels correlated with cognitive decline in advanced Alzheimer's patients (30). Interestingly, antioxidant supplementation might modulate GTP-CH1 activity, and several compounds, including resveratrol and vitamins C and E, may reduce neopterin synthesis (31, 32).

Increased INF-γ and neopterin levels in our frail subjects reflect GTP-CH1 pathway activity, while the decreased NO may be due to BH4 deficiency, that attenuates NOS activity. Elevated levels of INF-γ and neopterin levels increased the frailty risk, while elevated NO decreased the risk. Moreover, the aforementioned laboratory measures may be used as predictive biomarkers in frailty.

Acknowledgment

The authors declare no conflicts of interest.

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