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Role of Soluble Programmed Cell Death- 1 in Rheumatoid Arthritis Patients

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ABSTRACT

Background: A transmembrane glycoprotein called soluble programmed death-1 (sPD-1) is expressed on T cells and is secreted into synovial fluid and peripheral circulation by proteolytic cleavage of membrane-bound protein.

Objectives: Assessment of sPD-1 plasma levels in cases with rheumatoid arthritis (RA) and the link with disease activity, laboratory, and clinical parameters.

Methods: This research involved 20 active RA cases with DAS score (> 2.6 score), 20 patients in remission (≤ 2.6 score), and 20 apparently healthy individuals as control group. All studied groups were subjected to obtaining patient histories, doing clinical assessments, and performing laboratory tests in form of CBC, ESR, CRP, RF, and Ant-CCP. Measurements of sPD-1 plasma levels and correlations with clinical, laboratory, and disease activities were made.

Results: Regarding demographic information, there were no notable differences across RA patients and controls (P > 0.05). In comparing DAS score of the studied groups, there was a significant increase in active group than remission group. There was a significant increase of SPD-1 levels in active group compared to remission and control groups (P = 0.001), while it was not significant regarding remission and control groups (P=0.054). Positive associations were found between the sPDL-1 and the ESR, Anti-CCP, CRP, and DAS score.

Conclusion: Plasma sPD-1 in RA cases was significantly raised, and they correlate with DAS28, demonstrating that sPD-1 could be a useful indicator of the degree of RA disease activity. SPD-1 might be a new biomarker or target for RA immunomodulatory treatment.

Keywords: Soluble programmed death 1, DAS score, Disease activity; sPD-1; Rheumatoid arthritis.

INTRODUCTION

The most prevalent inflammatory arthritis is rheumatoid arthritis (RA), which affects nearly 0.5%–1% of the population with a mean annual incidence of 0.02%–0.05% [(1)].

Genetic, viral, environmental, and hormonal factors are among the many factors that contribute to the chronic immune response, which is essential to the pathogenesis of RA. In RA, the adaptive and innate immune systems are out of balance, which leads to exaggerated immunological responses [(2)].

For RA patients, in order to avoid bone deformation, preserve daily functioning, and reduce symptoms like pain and edema, medical therapy is used. This is mostly due to the usage of DMARDs, such as methotrexate, sulfasalazine, hydroxychloroquine, leflunomide and TNF-alpha inhibitors. These medications are often defined by their ability to slow or stop the advancement of joint deterioration, impairment of life quality, handicap, and disability-related incapacity to work, therefore completely blocking the whole disease progression. Pain may be managed with the use of analgesics [(3, 4)] .

The 55-kDa transmembrane protein known as programmed cell death-1 (PD-1) has a 24% similarity in amino acids to cytotoxic T lymphocyte antigen 4 [(5)]. PD-1 is an important component of the CD28 family, which is a crucial component of how T-cells are activated and how they react to other cells. Activation causes the PD-1 expression on T cells, natural killer T cells, macrophages and activated B cells [(6, 7)]. T-cell receptor-mediated activities such as the synthesis of interferon-gamma, tumour necrosis factor-alpha, and interleukin-2 are blocked by PD-1 [(8)]. One of the crucial factors in the development of the RA illness is the creation of autoantibodies [(9)].

Because T cells express the regulatory protein PD-1, this implies that these cells are a regulatory point of concern for regulating the stimulation of B memory cells that produce autoantibodies. Autoimmunity is significantly influenced by PD-1 and its ligands (PD-Ls), which are substantial immune system negative moderators [(10)]. PD-L1 and 2 are the two ligands of PD-1, that work together to send signals that stop the immune reaction. Cellular and humoral immunity are both impacted by PD-L1 and PD-L2, which have a negative modulatory function in the immune reaction. Inducible expression of PD-L2 occurs on the surface of macrophages, dendritic cells, and other cell types, although its expression is restricted in comparison with that of PD-L1. Also, human PD-L2 is present in the vascular endothelial cells [(10)].

In RA, sPD-1 inhibits the PD-1/PD-L1 inhibitory cascade and is linked to prolonged stimulation of self-reactive T cells, which furthers the development of the illness over time [(11)].

Anti-PD-L1 or anti-PD-1 treatment in individuals with different tumours has also been linked to an increase in autoimmune problems, including the relapse or aggravation of already present rheumatic illnesses and the new emergence of autoimmune diseases [(12)].

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This study's objective was to evaluate the sPD-1 plasma levels in cases who had RA and to link those levels with the laboratory, clinical, and disease activity parameters.

MATERIALS AND METHODS

This case-control research was conducted at a single center. Cases were chosen from Tanta University Hospitals' Rheumatology and Rehabilitation Department's Outpatient Clinic.

Forty adult cases fulfilling the RA classification according to the 2010 ACR-EULAR criteria [(13)]and twenty healthy participants of similar age and sex were involved in this case control research. Cases with RA were subdivided into 2 subgroups (Group IA: 20 cases with active RA with DAS28 more than 2.6 score, and group IB: 20 cases with RA with DAS28 less than or equal to 2.6 score that corresponds to remission.

Exclusion criteria: Patients with other rheumatic disorders, history of liver or renal disorders.

Clinical assessment:

Both demographic information and a comprehensive medication history were collected. Erythrocyte sedimentation rate (ESR) 1st hour, patient pain and global evaluations, and tender and swollen joint counts were used to quantify RA activity using the Disease Activity Score 28 (DAS-28) [(14)]. The degree of disease activity may be classified as being in remission (DAS28 ≤2.6), mild (2.6< DAS28 ≤3.2), intermediate (3.2< DAS28 ≤5.1), or severe (DAS28 >5.1).

Laboratory assessment:

Laboratory investigations were done in the clinical pathology department. Tanta University hospitals using Thermo Fisher Scientific Inc. Konelab™ / T Series apparatus. Routine laboratory assessment: (ESR by Westergren method, CRP, RF, and anti-CCP by ELISA). Measurement of sPD L-1 using ELISA technique: This kit uses sandwich-ELISA. Microelisa strip plate in kit was prepared using sPD-L1-specific antibodies.

The applicable Microelisa stripplate wells received standards or samples, which were subsequently combined with the indicated antibody. Each well got an HRP-conjugated sPD-L1-specific antibody. Free parts are removed while washing. Each well had TMB substrate added.

The only wells that displayed blue colouring before turning yellow when the stop solution was introduced were those containing sPD-L1 and the HRP-conjugated sPD-L1 antibody. Spectrophotometrically, optical density (OD) was calculated at 450 nm wavelength.

The value of the OD rose in a manner that was proportional and direct to the sPD-L1 concentration. In light of this, we were able to arrive at an estimate of the concentration of sPD-L1 by contrasting the OD of the sample with the standard curve.

Ethical Approval:

The study was approved by the Ethics Board of Tanta University and the patients were given all the information they need about the trial. Each participant in the research provided a signed, informed consent form. This study was conducted in compliance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for human subjects. Permission number 34239/11/20

Statistical analysis

With the help of SPSS version 22, data were statistically analysed and expressed as mean ± standard deviation (SD). For categorical variables, the chi-square test was employed to compare various groups. Student t-test: used to compare two groups under study for numerical variables that were distributed normally. For comparisons involving more than two groups and normally distributed quantitative variables, the F-test (ANOVA) was used. For pairwise comparisons, the Post Hoc test (Tukey) is used. Receiver operating characteristic curve (ROC) is produced by graphing sensitivity (TP) on the Y axis and 1-specificity (FP) on the X axis at various cut off points.

The diagnostic effectiveness of the test was shown by the area under the ROC curve. Areas with performances around 100% are the best for the test, while areas with performances over 50% are considered acceptable. The ROC curve also enables performance comparison between two tests. Sensitivity:

The test's ability to accurately detect ill people among a group that are "true positives." The proportion of unidentified case "false negatives" decreases with increasing sensitivity. Specificity: The test's ability to accurately rule out those who are clear of the illness, or "true negatives." There will be less "false positives" included the more strict the criteria. Positive Predictive Value (PPV):

The likelihood that a disease will be present in those who get positive findings from a diagnostic test. Negative Predictive Value (NPV): The likelihood that a patient's negative findings from a diagnostic test did not have the illness. Using the Pearson coefficient, two quantitative variables with normal distributions were correlated. Statistical significance was defined as P values ≤ 0.05.

RESULTS

Thirty-eight RA cases were females, with a mean age of 42.75 ± 11.31 in group IA, and 45.60 ± 9.0 in group IB. Regarding demographic information, there were no notable differences across RA cases and controls.

The mean DAS-28 score was 4.66 ± 0.73 in group IA, while it was 2.24 ± 0.29 in group IB, in comparing DAS score of the studied groups, there was

a significant increase in active group when compared to remission group.

Regarding ESR, and CRP levels of the studied groups, there was significant increase in group 1A when compared to group 1B (P= 0.001), significant increase in group 1A when compared to group 2 (P= 0.001) and no significant increase in group 1B when compared to group 2 (P= 0.244, 0.315 respectively.

There was a significant increase of sPD-1 levels in group 1A (active group) compared to group 1B (remission) and group 2 (control group) (P = 0.001), while it was not significant regarding group 1B (remission) when compared to group 2 (control group) (P=0.054). Table 1 provided information on the demographics, clinical data, and laboratory findings of RA patients and controls.

Table (1): Patient characteristics of the RA patients and controls

	Group IA (active RA) (20)	**Group IB (remission) (20)**	**Group II (control) (20)**	**Test of Sig.**	**P**
Age (years)	42.75 ± 11.31	45.60 ± 9.0	44.20 ± 8.64	F=0.430	0.653
Sex: (male/female)	1/19	1/19	2/18	X^2=0.53	0.765
Duration of RA (ys):	7.14 ± 2.43	5.69 ± 3.83	-	t=-0.44	0.66
Morning stiffness (min)	45.4 ± 10.75	15.2 ± 11.82	-	**t=8.45**	**0.001***
Tender joint count	6.51 ± 2.14	1.35 ± 1.09	-	**t=9.61**	**0.001***
Swollen joint count	3.75 ± 3.27	0.93 ± 1.41	-	**t=3.54**	**0.001***
DAS score	4.66 ± 0.73	2.24 ± 0.29	-	**t=13.738**	**0.001***
Treatment received: - **csDMARDs monotherapy** - **csDMARDs combined therapy** - **bDMARDs**	7 9 4	6 10 4		X^2=0. 0.13	0.93
ESR (mm/1st h)	40.05 ± 27.50	12.00 ± 4.42	6.00 ± 1.49	**F=25.480**	**<0.001***
CRP (mg/dl)	57.06 ± 27.91	8.97 ± 2.49	3.78 ± 1.76	**F=65.686**	**0.001***
Anti CCP (U/ml)	242.95 ± 42.24	42.49 ± 3.43	17.60 ± 3.70	**F=44.665**	**0.001***
RF	116.20 ± 100.97	32.07 ± 16.39	3.21 ± 1.61	**F=19.758**	**0.001***
S.PD-1 (pg /ml)	1305.81 ± 51.07	416.41 ± 17.86	225.51 ± 8.18	**F=70.810**	**0.001***

χ^2: Chi square test; F: ANOVA test; t: Student t-test; *: significant as $p \leq 0.05$.

There were positive correlations between sPDL-1 and ESR, Anti- CCP, CRP, and DAS score, while there was no correlation with RF (Table 2 and figure 1).

Table (2): Correlation between plasma level of S.PD-1 and different parameters in total cases group

	S.PD-1 (pg/ml)	
	r	**p**
Age	0.068	0.677
Rheumatoid factor	0.233	0.148
Anti CCP	**0.476**	**0.002***
ESR first hour	0.382	0.035
ESR Second hour	**0.608**	**0.001***
CRP	**0.654**	**0.001***
DAS 28 Score	**0.730**	**0.001***

*: significant as $p \leq 0.05$.

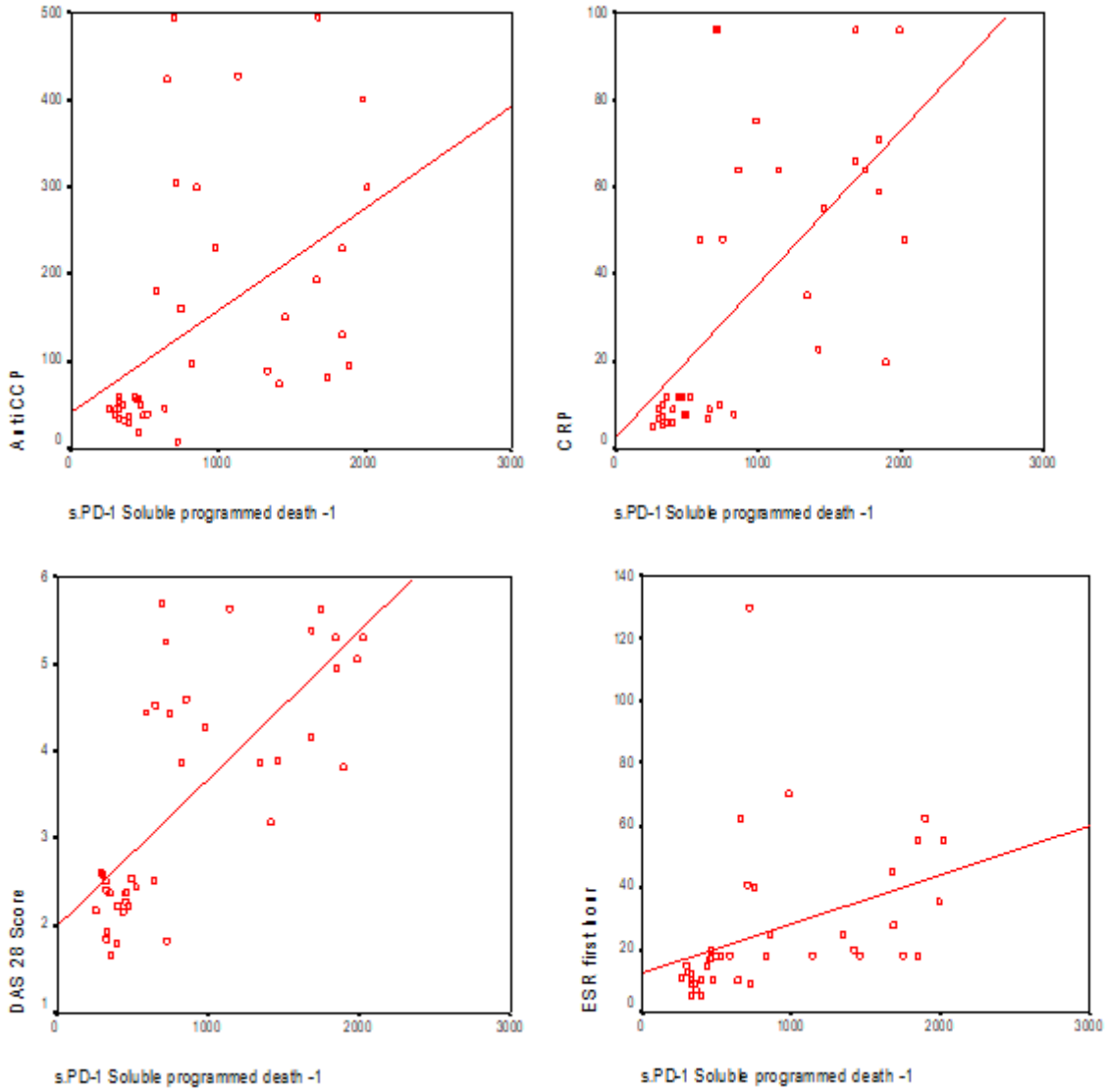

Figure (1): Correlation between plasma level of S. PD-1 demographic, clinical and laboratory characteristics of the studied groups.

Figure (2) showed ROC curve of sPD-1 level in group 1A &1B (active and remission) on comparison with control, sPD-1) level cut off was > 310 pg/ml, sensitivity 92.5%, specificity 80%, PPV 90%, NPV 84% and area under curve 0.962. The Linear regression for measurement of the sPD-1 level on different variables was presented in table (3). We found that 2nd hour ESR, CRP, and DAS-28 score were independently associated with slsv PD-1 levels.

Table (3): Linear regression for measurement of the soluble programmed cell death -1 (S.PD-1) level on different variables

	Univariate analysis		**Multivariate analysis**	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
Anti CCP	0.438 (0.293 – 0.743)	**0.018***	0.674 (0.419 – 2.316)	0.205
ESR first hour	0.418 (0.108 – 1.673)	0.103		
ESR Second hour	0.743 (0.397 – 0.912)	**0.001***	0.598 (0.274 – 0.763)	**0.021***
CRP	0.439 (0.234 – 0.675)	**0.001***	0.528 (0.369 – 0.823)	**0.017***
DAS 28 score	0.534 (0.138 – 0.864)	**0.001***	0.619 (0.276 – 0.903)	**0.008***

*: significant at $p \leq 0.05$

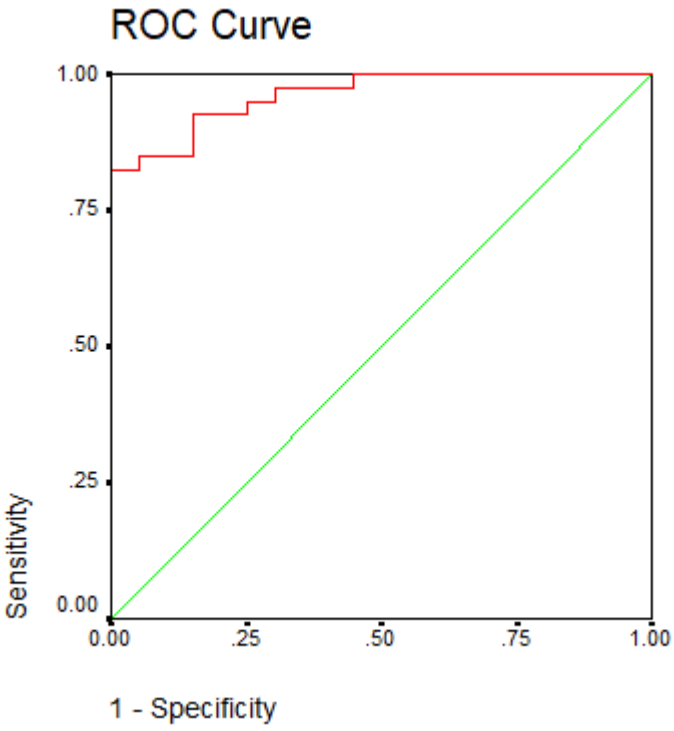

Figure (2): ROC curve of sPDL-1 levels in group 1A &1B (active and remission) on comparison with control.

DISCUSSION

An estimated 0.5% to 1% of the population suffers with RA, making it one of the most prevalent inflammatory autoimmune diseases. It often develops after the age of 50, and women are afflicted three to four times more than males. Patients with some malignancies and autoimmune illnesses have higher sPD-L1 levels, which are strongly associated with negative outcomes [(7)].

Clinical and serological measurements may be used to forecast the emergence of established RA in individuals who are still in the early stages of the illness [(15)]. Therefore, a deeper comprehension of the disease processes and biomarkers at this initial stage would be crucial for identifying potential novel therapeutic approaches and for customizing therapy to guarantee the best possible care for each patient [(17)].

In this study female gender represented 95% of the patients' group, this was reported by **Mansour *et al.*** [(18)] and **Wang and Liang** [(12)] as they found that there is a female predominance in their study.

As regards sPD-1, there was a significant increase of its levels in active group than remission and control groups while not significant regarding remission and control groups. This is in agreement with Hassan **WA *et al.*** [(6)] who showed that plasma level of sPD-L1 elevated in RA patients than healthy control, and sPD-1 levels were shown to be highly associated to disease activity and the positivity of the RF.

Important data on a patient's current disease activity is obtained through laboratory testing. Acute phase reactants, such as ESR and CRP, which are increased in the majority of RA patients, are often used to evaluate the level of activation. These indicators make up a number of composite indices and might possibly be used as an entry requirement for clinical studies [(18)]. Nevertheless, it has been noted that around 50% of RA patients have normal ESR or CRP, and some researchers speculate that this figure may even be overestimated. Additionally, in most but not all RA patients, a decrease in CRP or ESR indicates an improvement clinically. In actuality, these acute phase biomarkers could stay the equivalent in certain populations and might not be accurate indicators of inflammatory activity. Therefore, in a large proportion of individuals, actual disease activity may go undiagnosed by commonly used laboratory testing [(19)].

The results of this investigation indicated that there was a difference that had been statistically significant between the two groups. It indicated a rise in plasma Anti-CCP levels in the active group, when contrasted with the remission and the control groups. On the other hand, the difference between the remission and control groups was not statistically significant.

This is in accordance with the findings of **Mansour *et al.*** [(17)] who demonstrated a statistically significant rise in the quantity of anti-CCP in the serum of RA patients in comparison with healthy control groups.

The findings of **Wang and Liang,** [(12)], which aligned with the findings of the present investigation, indicated that patients with active illness had greater

levels of anti-CCP than patients who have established remission from their condition. The levels of anti-CCP may be used to forecast the advancement of RA in individuals who currently have early and nonspecific arthritis, the level of activity in cases who currently have established RA, and the likelihood of developing RA in the prospective in specific high-risk groups are all things that need to be taken into consideration [(20)]. Anti-CCP, along with RF, is included in the newly established diagnostic criteria for RA, which has just been implemented [(21)]. Additionally, we discovered a significant statistical link between plasma sPD-1 levels and the ESR, CRP level, RF titers, anti-CCP titers, and DAS28 scores. According to the significant link between sPD-1 and DAS28, sPD-1 may serve as a measure of activity of the disease. Our findings supported those of previous researchers who discovered elevated plasma sPD-1 concentrations in RA patients than controls [(22)].

Due to the substantial association between the PD-1 gene and RA susceptibility and the fact that inflamed synovium is at least partially where circulating sPD-1 is produced [(11)], it has been hypothesized that PD-1 may contribute to RA pathogenesis [(23)]. Higher concentrations of sPD-1 were discovered by **Cuiping *et al.*** [(24)] in the synovial fluid and sera of RA cases. According to **Li *et al.*** [(25)], elevated blood sPD-1 levels might be a useful biomarker for RA cases who have ILD since they indicate the existence of the condition. However, a research by **Wang *et al.*** [(26)] found no connection between sPD-1 ligand gene polymorphisms and RA susceptibility.

PD-L1 and 2 are the two ligands of PD-1, that work together to send signals that stop the immune reaction. Cellular and humoral immunity are both impacted by PD-L1 and PD-L2, which have a negative modulatory function in the immune reaction. When sPD-1 relates with PD-Ls, it blocks the PD-1/PD-L signaling pathway, which makes T cells more active [(27)]. The area of oncology has shown significant success with therapeutic anti-PD-1/PD-L1 antibodies. Clinical investigations in people and experimental mice models have provided strong evidence that the signaling pathway of the PD-1 has a role in mechanism of numerous chronic inflammatory disorders, including RA [(28,29)].

Both negative and positive co-stimulatory factors exert a significant amount of control on the activation and action of T cells. In autoimmune disorders like RA, abnormal co-stimulatory molecule production and activity have been linked to self-reactive T cells that remain permanently activated [(24)]. It is well established that sPD-1 and its ligand have a critical role in immunoregulation by regulating the advancement of effector immune cells [(30)]. The role of sPD-1 is to block and inhibit membrane-bound PD1's immune regulatory effect on T cell activation, which exacerbates the disease. However, some have suggested that sPD-1 performs similarly to membrane-bound PD1, demonstrating immune regulation by restricting TCR-induced processes [(31)].

This research had certain limitations since it was conducted in a single location. It would have been preferable if the participants had been more numerous, and the study had been conducted in other locations. Future longitudinal studies are required to determine the relationship between sPD-1 and disease severity and progression as our research is cross-sectional in nature.

CONCLUSION

Plasma sPD-1 concentrations in RA patients were considerably higher than normal, and they had been linked with DAS28. This suggests that sPD-1 may be a helpful measure for RA disease activity. Hence, sPD-1 could be a useful marker or target for the immunomodulatory therapy of RA.

DECLARATIONS

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- **Consent for publication:** I attest that all authors have agreed to submit the work.
- **Availability of data and material:** Available
- **Competing interests:** None
- **Funding:** No fund
- **Conflicts of interest:** no conflicts of interest.

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