



Association between Indoor Environment, Blood Trace Elements, and Immune Globulin among Workers from Vegetables Plastic Greenhouses in Yinchuan, China

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Abstract

Background: To study the association between indoor environment (MiE), blood trace elements (BTE), and immune globulin (PRO) among workers from vegetables plastic greenhouse, and to assess the mediate effects on MiE and PRO by BTE.

Methods: Overall, 168 practitioner and corresponding sheds were included from cross-sectional study in 2016. BTE and PRO were determined by physical test and MiE data from field and laboratory measurement. The association was assessed using canonical correlation analysis. The direct and indirect effects between MiE, PRO and BTE were conducted by structural equation model. 5000 times bootstrap methods were performed to estimate coefficient and 95% confidence interval.

Results: MiE was moderately correlated with BTE (canonical coefficient = 0.439), and BTE was strongly correlated with PRO (canonical coefficient = 0.514 and 0.481). No statistical evidence was found for the overall impact of MiE on PRO, and BTE as an intermediary affecting its relevance was not confirmed. Only the path way of the BTE impact on PRO had a significant positive effect ($P=0.012$).

Conclusion: BTE was positively associated with PRO, therefore, reducing exposure in greenhouse is a pathway to remain blood trace elements, and further effect the immune protein in human body.

Keywords: Plastic greenhouse; Microenvironment; Body trace element; Immune globulin

Introduction

Greenhouse is a major type of agriculture covering the world. It can achieve higher crop production by modifying the natural environment, espe-

cially prolonging the growth season compared with that in the open field (1). In Northwest China, the type of plastic greenhouse has been wide-



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ly used. Nowadays, China has the highest greenhouse-based vegetable production system in the world, with an estimated amount of more than 4 million hm² in 2015 (2) due to the population growth and accelerated urbanization (3).

Vegetable production in greenhouses needs pesticides, high temperature and relative humidity to ensure the increase of production (4). As far as we know, the microenvironment in vegetable greenhouse also has an influence on health, but there is rarely research on it. The characteristics of the indoor environment are as follows: 1. In most cases, workers have to experience cold and heat, wet and dry environment many times in each working day, which may have an adverse impact on health. 2. Moderately high temperature and humidity exposure for a long time is also a hazards factor. A lot of publications show that environment in the greenhouse increases the incidence rate of respiratory and cardiovascular diseases (5-8), but few research mentioned mechanism about it. Another important feature of vegetable greenhouses is high nitrogen oxides (NO₂) and sulfur dioxide (SO₂) exposure (9,10), which has been proved as toxic substance (11). In the greenhouse, NO₂ and SO₂ are always accompanied by high temperature and humidity, they are all non-independent.

This study focused on evaluating the association between microenvironment, blood trace elements and immune globulin in vegetable greenhouse workers. The blood trace elements was also assessed to determine whether they have mediation effect on relationship between microenvironment and blood protein.

Materials and Methods

Sample preparation

The data of plastic vegetable greenhouse microenvironment (MiE) was collected from a cross-section field survey from April to May in 2016. Forty-six greenhouses were selected from four villages (Liangtian, Maosheng, Wudu, Yinhe) on the outskirts of Yinchuan City, Ningxia Province, China.

The study was approved by the Ethics Committee of Ningxia Medical University (2014-090). All participants signed written informed consent. The physical examination data were collected from 168 greenhouse workers. The blood sample were tested in clinical laboratory of Yinchuan Guotai Hospital by clinician. Physical examination was carried out from July 13 to 15, 2016. Physical examination items include blood trace elements (BTE, include magnesium, inorganic phosphorus, cupric ion, calcium ion, zinc ion and potassium ion) and immune globulin indicators (PRO, immunoglobulin G, immunoglobulin M, immunoglobulin A).

Study criterion

The standard for selecting vegetable greenhouse include: 1) The material of greenhouse was plastic; 2) Used for planting vegetables for more than five years; 3) Plant species were only vegetables; 4) The greenhouse was used continuously in the past five years; 5) There was no turnover of practitioners in greenhouse cultivation.

The standard for selecting workers include: 1) Local resident (at least five years); 2) Engaged in vegetables greenhouse cultivation for at least five years; 3) without medication history in the last year; 4) No history of heart disease; 5) Usually spraying pesticides to take protective measures.

Detection of Indicators of indoor environment

The indicator of temperature, humidity, nitrogen oxides and sulfur dioxide were collected through air sample. HM 34 HUMIDITY &TEMPERATURE METER (Vaisala Co.Ltd. Finland) was used to detect temperature and humidity. Temperature and humidity data were collected from three points in each shed, including near, middle and far distance from the gate. Air sample were collected using QC-4S explosion-proof atmosphere sampler (Beijing institute of labor protection science, China). Three-dimensional sampling of the median line was used to collect nitrogen oxides and sulfur dioxide data. Two monitoring points were set, two at-

atmospheric samplers were placed in each point. At the same time, a sampling point was set out of the greenhouse as control. Previous studies were referred for the methods of detecting sulfur dioxide and nitrogen oxide.

Detection of blood trace elements and blood immune protein

BTE and PRO were detected by XS-500i automatic five count blood cell counter (SYSMEX Co.Ltd. Japan) and MS-880 automatic biochemical analyzer (Medical System Biotechnology Co., Ltd. Ningbo, China). All the test procedures were performed in the Yinchuan Guotai hospital by clinician. The test sample was the fasting blood of the workers.

Statistical Analysis

The health impact factor and body indexes were dependent. The inter association between three sets of variables (MiE, BTE and PRO) were detected using canonical correlations analysis (CCA), which was derived from two canonical variants of shared variance as opposed to two variables in a traditional Pearson or Spearman correlation analysis (12).

The mediate effect was demonstrated by structural equation model (SEM). We hypothesized

that BTE has a mediation effect on association between MiE and PRO. Then the mediated effect was estimated by 5000 times bootstrap distribution method (13).

All data were processed using SPSS 24.0 (IBM Corp., Armonk, NY, USA) and AMOS 22.0.

Path Diagram

The hypothesis of relationship among elements in current study is shown in Fig. 1. Basic medicine research evidence suggested that the body trace elements could be worked in synergy to support the protective activities of the immune cells (14-16). Microenvironment might have several ways to directly impact BTE concentration, like skin touch, oral and respiratory system. Then assume that these two sets directly influence were one-way single direct arrow line. There is few literature about the natural environment impact on the human immune globulin synthesis, but some research revealed that climate change can interrupt structure of fish's protein product (17). There is a simple logic assumption that MiE would be effective on immune globulin. Lastly, it could be supposed that mediation effects of BTE can exist based on above theory, BTE would be set as a mediator.

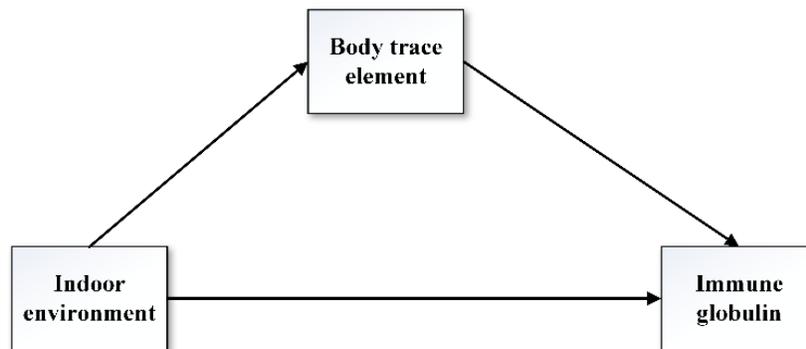


Fig. 1: The hypothesized model for the relationships among Indoor environment, Body trace element and immune globulin

Results

The distribution patterns of four statistic of MiE and ten physical indexes were studied. The basic statistics are presented in Table 1. The exposure

time in plastic greenhouse of vegetables was collected through questionnaire. The results showed that the workers usually spend about 7.5 hours in sheds in spring, summer and autumn, 6.5 hours in winter.

Table 1: Descriptive statistics of greenhouse Indoor environment and physical variables for all 168 samples

<i>Variables</i>	<i>Mean(SD)</i>	<i>±95%CI</i>	<i>Min- Max</i>	<i>Median(IQR)</i>
<i>Indoor environment</i>				
Relative Humidity %	59.83(17.95)	57.09-62.56	31.93-95.57	55.25(44.04-77.93)
Temperature °C	25.58(2.89)	25.15-26.02	18.37-32.67	25.20(23.97-27.73)
NO ₂ mg/m ³	0.04(0.02)	0.03-0.04	0.00-0.10	0.04(0.02-0.05)
SO ₂ mg/m ³	0.05(0.04)	0.04-0.05	0.01-0.17	0.03(0.02-0.06)
<i>Body trace element</i>				
Mg ⁺² mEq/L	1.20(0.05)	1.19-1.20	1.00-1.28	1.20(1.19-1.21)
PHOS mEq/L	1.10(0.09)	1.09-1.12	0.70-1.34	1.10(1.09-1.12)
Cu ⁺² mEq/L	15.38(2.02)	15.07-15.69	11.02-30.12	15.32(14.81-15.92)
Ca ⁺² mEq/L	2.50(1.44)	2.28-2.72	2.00-15.65	2.44(2.17-2.51)
Zn ⁺² mEq/L	14.18(1.81)	13.91-14.46	0.80-19.20	14.25(14.00-14.62)
K ⁺ mEq/L	6.64(2.54)	6.25-7.03	2.01-14.80	6.57(4.21-8.93)
<i>Immune globulin</i>				
IgG	15.88(3.67)	15.32-16.44	0.30-28.20	15.70(14.01-17.00)
IgM	2.28(8.70)	0.96-3.61	0.56-114.00	1.46(1.10-1.95)
IgA	16.23(159.60)	-8.08-40.54	1.07-2072.00	3.15(2.44-3.86)

The association between MiE, BTE and PRO were demonstrated by bivariate correlations analysis, as shown in Table 2. The correlation within

the group was statistically significantly stronger than that between groups, ranging from weak to moderate (the absolute value from 0.01 to 0.468).

Table 2: Bivariate Correlations Between the study variables

	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>	<i>8</i>	<i>9</i>	<i>10</i>	<i>11</i>	<i>12</i>	<i>13</i>
1 Relative Humidity	1												
2 Temperature	-.336**	1											
3 NO _x	-.147	.083	1										
4 SO ₂	-.199**	.080	.295**	1									
5 Mg ⁺²	.032	.061	-.022	-.044	1								
6 PHOS	.007	.128	.072	.034	.044	1							
7 Cu ⁺²	.138	-.119	-.178*	-.193*	-.011	-.160*	1						
8 Ca ⁺²	.082	-.034	-.066	-.019	.007	.002	-.110	1					
9 Zn ⁺²	.063	-.100	.010	.004	.032	-.011	.193*	-.813**	1				
10 K ⁺	-.279**	.198*	.207**	.120	.077	.064	-.083	-.102	-.001	1			
11 IgG	.039	.122	.065	.067	.063	.103	.021	-.468**	.323**	.082	1		
12 IgM	-.001	.180*	.037	-.035	.081	.182*	.037	.019	-.215**	-.060	.244**	1	
13 IgA	-.093	.061	-.066	-.066	.086	.118	-.105	-.027	-.081	-.076	-.018	-.011	1

** . Correlation is significant at the 0.01 level (2-tailed). * . Correlation is significant at the 0.05 level (2-tailed).

Canonical correlations were extracted, the results of canonical correlation are displayed in Table 3 and 4. Panel I in Table 3 shows the standard canonical correlation with MiE and BTE. Only the

first canonical correlation has statistics significant and the value was 0.439, which represented a medium effect. Panel II displays the relationship

between BTE and PRO, the first two correlations remained significant and the r value was 0.514 and 0.481 respectively, representing a large-sized and medium effect. That also support the relationship that Panel II was stronger than Panel I. All of the canonical correlations of MiE and PRO were not statistically significant. Fig. 2 illustrates the standard results of the structural equation model. Standardized path coeffi-

cient of the MiE to PRO was -0.03 ($P>0.05$), MiE to BTE was 0.16, BTE to PRO was 0.25 ($P=0.012$), only path BTE to PRO has statistically significance. MiE's impact on PRO was negative but not significant. MiE's impact on BTE was not significant and exerts positively effect. The data at hand did not unequivocally support that there is mediate effect of BTE on MiE to PRO (data not showed).

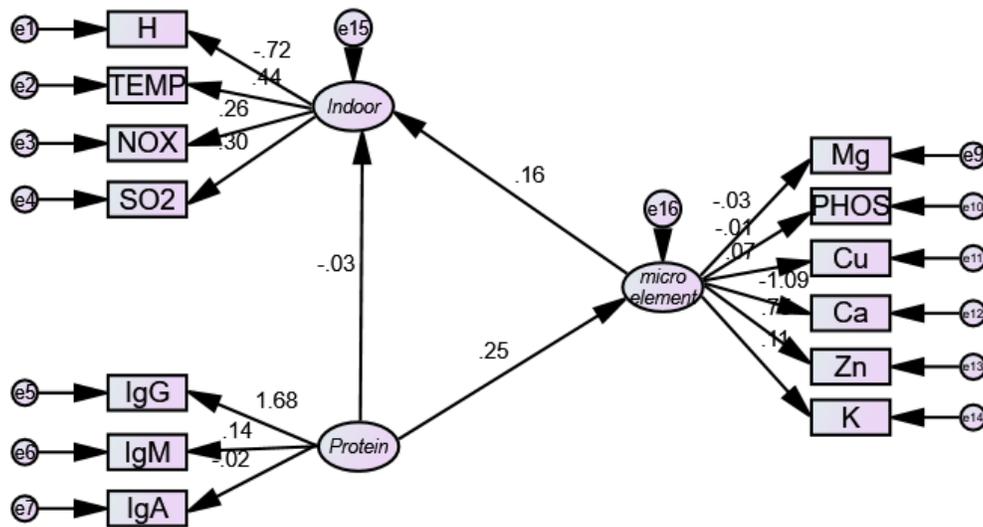


Fig. 2: Path Diagram and Standardized Results of Modified Structure Equation Modeling(MSEM) analysis. Note. H=Relative Humidity, TEMP=Temperature, NOX=oxynitride, SO2=sulfur dioxide, Indoor=Indoor environment, IgG=immunoglobulin G, IgM=immunoglobulin M, IgA=immunoglobulin A, Mg=magnesium, PHOS=inorganic phosphorus, Cu=cupric ion, Ca=calcium ion, Zn=zinc ion, K=potassium ion, micro element=Body trace element, e=measured error. Circle=latent variable, Rectangle=measured variable
Model fitting indices: $\chi^2/df=2.199$; RMSEA=0.085; 90%CI for RMSEA: 0.065-0.104; GFI=0.890; AGFI=0.838

Table 3: The results of the canonical correlation analysis of MiE, BTE and PRO

	<i>Correlation</i>	<i>Eigenvalue</i>	<i>Wilks Statistic</i>	<i>F</i>	<i>P</i>
Panel I (MiE vs. BTE)					
1	0.439	0.238	0.756	1.923	0.006
2	0.200	0.042	0.936	0.710	0.775
3	0.151	0.023	0.975	0.510	0.849
4	0.048	0.002	0.998	0.125	0.945
Panel II (BTE vs. PRO)					
1	0.514	0.358	0.556	5.765	<0.001
2	0.481	0.301	0.755	4.819	<0.001
3	0.130	0.017	0.983	0.697	0.595

Note: H_0 for Wilks test is that the correlations in the current and following rows are zero

Table 4: Standardized Canonical Coefficients and Canonical Loadings for MiE, BTE and PRO 1, 2, 3 and 4

Variable	1			2			3			4		
	Coef	Load- ing	Cload- ing									
Panel I (MiE vs. BTE)												
<i>MiE</i>												
Relative Hu- midity	-	-0.786	-0.345	0.027	0.070	0.014	-	-0.576	-0.087	-	-0.213	-0.010
Temperature	0.563	0.635	0.279	-	-0.587	-0.117	0.916	-	-0.067	0.130	0.237	0.011
NO _x	0.400	0.586	0.257	0.657	0.430	0.086	0.703	-	-0.257	-	-0.637	-0.031
SO ₂	0.434	0.395	0.173	0.286	0.713	0.143	0.231	-	-0.293	0.885	0.500	0.024
	0.123			0.687			0.350			0.718		
<i>BTE</i>												
Mg ⁺²	-	-0.020	-0.009	-	-0.381	-0.076	-	-0.346	-0.052	-	-0.080	-0.004
PHOS	0.052	0.188	0.082	0.431	-0.200	-0.040	0.313	-	-0.827	0.008	-0.290	-0.014
Cu ⁺²	0.077	-0.515	-0.226	0.286	-0.506	-0.101	0.776	0.436	0.066	0.343	-0.500	-0.024
Ca ⁺²	-	-0.207	-0.091	-	-0.036	-0.007	0.348	-0.191	-0.029	0.457	0.554	0.027
Zn ⁺²	0.398	-0.161	-0.071	0.688	0.364	0.073	0.386	0.058	0.009	0.211	-0.736	-0.035
K ⁺	-	0.777	0.341	0.934	0.022	0.004	0.321	0.178	0.027	0.823	-0.409	-0.020
	0.733			0.113			0.241			0.447		
Panel II (BTE vs. PRO)												
<i>BTE</i>												
Mg ⁺²	0.262	0.221	0.114	0.150	0.100	0.048	0.259	0.246	0.032	-	-	-
PHOS	0.381	0.381	0.196	0.267	0.237	0.114	-	0.070	0.009	-	-	-
Cu ⁺²	0.051	-0.025	-0.013	0.177	-0.031	-0.015	0.059	-0.844	-0.110	-	-	-
Ca ⁺²	-	-0.684	-0.351	-	0.706	0.340	0.926	0.189	0.074	0.010	-	-
Zn ⁺²	1.526	0.239	0.123	0.198	-0.898	-0.432	0.321	-0.002	0.000	-	-	-
K ⁺	-	0.013	0.007	-	-0.272	-0.131	-	-0.346	-0.045	-	-	-
	0.184			0.307			0.420					
<i>PRO</i>												
IgG	0.744	0.832	0.427	-	-0.510	-0.245	-	-0.217	-0.028	-	-	-
IgM	0.392	0.569	0.292	0.709	0.659	0.317	0.089	-0.492	-0.064	-	-	-
IgA	0.406	0.388	0.199	0.835	0.298	0.143	0.460	0.865	0.872	0.114	-	-

Note: Coef= Standardized Canonical Correlation Coefficients; Loading= Canonical Structure Coefficient; CLoading= Cross-structure Correlation

Discussion

The results of this study clearly indicate that MiE is associated with organism biochemical indexes. Canonical analysis was used to establish a relationship pathway among the three variables sets,

taking into account that most workers spend nearly a third of their time in the greenhouse per day. Specifically, MiE has medium-strength association with BTE and the coefficient was 0.439 (18), which represent that high humidity could reduce concentration of potassium and increase other four elements. The roles of the other three

indoor environmental indicators were opposite to humidity, and sulfur dioxide showed the weakest correlation. There is rarely direct evidence to explain how humidity changes potassium in human body. High humidity always comes with a high temperature in the greenhouse, resulting in the acceleration of human metabolism, and then magnesium ions are eliminated with the rapid discharge of body fluid. But there is a conflict point. Temperature and humidity have the opposite effect on all research body blood ions. The potential interactions could exist among the measurement variables (19-21), so statistical analysis was performed on the relative indicators as a whole rather than fragmentation.

Similarly, BTE has a strong positive correlation with PRO. The standardized path coefficient was 0.25, which was statistically significant, indicating that trace elements could affect the immunoglobulin as effectively as previous research evidences (22-26). The canonical coefficients is statistically significant, indicating that the role of most elements is the same as the previous results (27-29). Cu was a confirmed cause to elevate the risk of cardiovascular disease (30). Potassium effect on PRO mechanisms might be that it plays a role as competitor to inhibit Ca^{2+} channel on cell membrane and could dysfunction immune protein. The second canonical coefficient indicated that Mg^{+2} , PHOS and Cu^{2+} had a negative effect on immunoglobulin in the medium related to BTE and PRO, while the rest three indexes played an activator to immune protein in the practitioners of vegetable greenhouse. The rank of effect size to immunoglobulin was IgM, IgG and IgA, and contribution degree of BTE follow as Zn^{+2} , K^+ , PHOS, Ca^{+2} , Cu^{2+} , Mg^{2+} . The results showed opposite direction and contradiction compared with common sense perception of zinc effect immune functions (31,32). The reasonable cause could be that most of workers have zinc overdose through oral and skin exposed because they all spend more than seven hours in sheds. Overuse of pesticides and fertilizer could be increased risk of zinc exposure (33), and zinc was necessary element in most types of pesticide.

Immunoglobulins are the basic organics substances that protect our bodies throughout all life (34,35). As the farmer in a greenhouse, MiE may affect protein synthesis in a chronic process, even though this study found no evidence. Consider that there is a stronger potential interaction, it was more necessary to integrate indoor environmental indicators, body blood trace elements indexes and body four protein indexes into a latent variable respectively. The total effect about MiE on PRO and mediate effect were not found in this research, the results does not necessarily means that PRO is unaffected by such schemes. The most likely cause could be considered that the formation of immune protein is more complicated than the intake of trace elements, the lag effect of environmental impact on organism health and the adaptability of body regulation to environmental change (36-38). The hypothesis of this study that BTE is set as an intermediary variable affecting MiE and PRO, has not been verified. The cross-sectional data could not determine this long-term effect. This lag effect may be masked, and it may has difficulty to identify these effects.

There are some limitations in this study. First, mediation analysis should perfectly be carried out using longitudinal data, which will lead to a confusion where BTE affect PRO or PRO regulation and affect the BTE. If there were evidence of causality, cohort studies could be done more efficiently. Therefore, to best capture the causality, this study would have benefited from a longer-term indoor environment and bio-indicator monitoring. Secondly, the lack of specific pesticide information collection is the biggest shortcoming, which will produce possible confounding factors, including a reasonable interpretation of the results in this research. Although no pesticide information was used, the inter linkages between each information was utilized, thus the lack of pesticide information can be avoided. Another limitation of this study is that we performed one mediation mechanism in which MiE affects PRO, whereas the relationship like as environment and human health always is undoubtedly multi-factorial.

Conclusion

This study revealed the influence and intensity of MiE on the BTE of vegetable practitioners, as well as the influence and strength of BTE on PRO. It advised that reducing greenhouse exposure was an effective way to remain blood trace elements and further impact immune protein. Further work that builds on this conclusion will allow for the development of healthy policies to help management, and reduce the adverse health effects of vegetables plastic greenhouse work. The microenvironment affecting the mechanism of the protein can be elucidated, as well as the impact of the mediator effect and the regulation of the effect of the determination of indicators.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interest.

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