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ORIGINAL ARTICLE

Analyses of Trace Elements, Arsenic (As), Boron (B), Lead (Pb), Strontium (Sr) and Zinc (Zn), in Human Body and their Correlation with Immune Status Against Viral Infections

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ABSTRACT

Background: Trace elements have been used since ancient times as a therapeutic agent. However, until recently its use in modern medicine has been restricted to the treatment of a limited number of parasitic infections, but now some evidence is there that they can be helpful in the treatment of a variety of diseases.

Aim and Objective: This research was organized to investigate the concentration of trace metals in human blood and their positive effects against viral infection.

Methods: Instrumental analysis of the elevated level of metal concentration was performed by Inductively Coupled plasma-optical emission spectroscopy (ICP-OES) and the presence of virus antibodies by Enzyme linked immunosorbent assay (ELISA) and viral load analysis by Real time Polymerase chain reaction (RT-PCR).

Results: The Mean±SD of As, B, Pb, Sr and Zn in negative RT-PCR samples of H1N1 were (141.9±47.3), (417.6±119.3), (149.1±37.5), (6.2±1.6), (535.6±104.7) ppb respectively, the Mean±SD of As, B, Pb, Sr and Zn in negative RT-PCR samples of HIV were (12.5±0.9), (169.8±35.2), (138.2±34.5), (8.12±1.3), (421.4±40.1) ppb respectively, the Mean±SD of As, B, Pb, Sr and Zn in negative RT-PCR samples of HBV were (122.3±21.6), (160.7±23.6), (40.9±8.5), (53.7±7.7), (1459.4±90.1) ppb respectively, the Mean±SD of As, B, Pb, Sr and Zn in negative RT-PCR samples of HCV were (4.9±1.8), (3.7±1.34), (123.2±31.7), (60.9±22.5), (180.2±55.6) ppb respectively. The As, B, Pb, Sr, Zn concentrations in all the RT PCR negative samples were above the permissible limit, and also, we found a positive correlation between As levels and viral immunity in HCV patients (≤0.05).

Conclusion: These results indicated that an accumulation of metals, especially As, in the body for a long time provides immunity against many viral diseases (H1N1, HIV, HBV, and HCV).

Keywords: Immune status, Immune Response, Toxicity, Metals

INTRODUCTION

The immune system is based upon a complex network of cells that perform function in coordination to save the body from the invading organisms[1]. An immunocompetent system protects the host against illness, inflammation, contaminants (biological or not), or other undesirable infiltrations[2]. Viruses are toxic agents that proliferate in living cells of humans[3]. Each virus consists of genetic material and proteins that both assist in its replication in a host, but ultimately also may provide the means by which the host can develop immunity against virus[4].

The field of immunotoxicology is in its inception, but within the past few years appreciable information has been accomplished on many chemicals in different species of animals by promoting a spread of immunoassays. But the information that has been collected often doesn't recognize the whole immunologic response and further insights are required to elaborate the role of trace elements in boosting immunity against viral diseases[5].

Many immunoassays have a comparatively low amount of sensitivity index and, thus, require appreciable enhancement to analyze the immune status of an individual. Ideally, it would be beneficial to work out that which chemicals renders human cells more prone to toxic agents or retard antibody titers[6]. The effect of the chemicals on the activity of B and T lymphocytes, macrophages, their soluble compound should be analyzed directly[7]. Reduction of humoral immunity doesn't automatically encourage lymph cell involvement, after all T cells, like helper, suppressor, or cytotoxic functional subclasses, could also be afflicted individually or collectively.

Lymphokines and soluble factors from lymphocytes and macrophages regulate immunity[8] So, it has been established that a metal interferes with the immune reaction of host, every effort should be made to regulate the particular mechanism by which that compound alters the response, whether it is cellular or sub cellular and if it involves one or many components of the system[9,10].

In other types of toxicities, immune cells themselves are impacted by the metals. These types of changes can be seen as alterations in absolute numbers of given immune cell types, formation/release of key immunoregulating proteins (i.e., cytokines, chemokines, etc.), and/or shifting of regulatory patterns (i.e., T-helper-1 vs. TH2) in the host. In all cases, these induced alterations can ultimately manifest as changes in a host ability to recognize and then remove a viral/bacterial challenge. In all cases, these induced alterations can ultimately manifest as changes in a host ability to recognize and then remove a viral/bacterial challenge[11,12].

The investigation and identification of the level of metals in a sample can be done through ICP-OES, In laboratories hepatitis B, hepatitis C, HIV and influenza virus is detected by distinct procedures most ordinary analytical techniques of screening are depend on immunochromatographic analysis (ICT), ELISA and PCR[13]. PCR diagnostic techniques are costly and are used in well equipped laboratories and main tertian health care centers. Rapid examination ICT kits are a well choice as they are less expensive and do not demand a high technical framework[14].

MATERIAL AND METHODS

Study Design: This cross-sectional study included 67 patients from Jinnah Hospital Lahore. After taking patients consent, 5ml of blood sample was drawn from median cubical vein in blood collection tube. After centrifugation 1ml serum was separated in sterile tube and stored at -20^{o}C for further serological tests and in the remaining sample we added nitric acid for storage of metals. Whole blood sample was also collected for DNA extraction and further PCR processing.

Screening for HBV, HCV, HIV: Samples were isolated for the detection of HBV, HCV, and HIV using an immunochromatographic method (ICT) rapid test kit. In ICT device lamina is coated with viral antibodies. Virus antigen display in the serum of patients' proceeds with the fragment laminated with antibody. The reaction assembles a colored line which emigrates upward on the membrane. In the region the existence of this colored line indicates positive

conclusion, and the absence of color line admitted negative results.

Enzyme linked immunosorbent assay (ELISA) for HBV, HCV, HIV and H1N1: Samples were further processed for HBV, HCV, HIV and H1N1 ELISA for the identification of viral antigen present in patient serum by sandwich ELISA technique. Serum was added in the wells precoated with antibodies and then enzyme conjugated was added. Finally, substrate was added, and absorbance was taken by ELSA plate reader at 450 nm.

DNA extraction for HBsAg, HCV, HIV and H1N1: All samples were processed for the extraction of DNA. For extraction of DNA we used the QIAamp DSP virus spin kit was used for the extraction of DNA.

Amplification of DNA OR RNA through Real time Polymerase chain reaction (RT-PCR): Amplification of extracted DNA was done by RT PCR under standard condition using the gene specific primer of HBsAg, HCV, HIV and H1N1.

Estimation of Metals content in the samples: Only 25 RT PCR negative samples of HIV, HBV, HCV and H1N1 infection were further processed for the estimation of metal content by ICP-OES method using wet acid digestion technique.

Statistical analysis: Data was entered and analyzed by SPSS 16. All data was reported as means ± SD. Data for metal contents were analyzed using an independent sample t-test. Pearson correlation was used to find the correlation between metal level and immune status.

RESULTS

Patient characteristics: A total number of 67 seropositive viral samples which included 12 hepatitis B virus (HBV), 45 hepatitis C virus (HCV), 5 Human immunodeficiency virus (HIV) and 5 influenza A virus (H1NI) were included in this study (Table 1)

Table 1: Participants in this study (n = 67), RT-PCR Negative 25

HBV (ICT and ELISA)	Total HBV cases	12
	Positive	12 (100%)
	Negative	0 (0%)
HCV (ICT and ELISA)	Total HCV cases	45
	Positive	45 (100%)
	Negative	0 (0%)
HIV (ICT and ELISA)	Total HIV cases	5
	Positive	5 (100%)
	Negative	0 (100%)
H1N1 (ELISA)	TotalH1N1 cases	5
	Positive	5 (100%)
	Negative	0 (0%)
Total Participants		67
HBV PCR	Negative	4
HCV PCR	Negative	17
HIV PCR	Negative	2
H1N1 PCR	Negative	2
RT PCR Negative Participants		25

All cases were tested for analysis of hepatitis B, hepatitis C and HIV on ICT test device. For further confirmation all these seropositive samples were tested through ELISA for detection of the presences of antigen of H1N1, HIV, HBV, and HCV. All cases were positive. All these seropositive samples tested for presence of DNA/RNA through RT-PCR. 25 cases were negative these negative cases included in this investigation and further 42 positive RT-PCR cases were excluded.

Determination of Elements by ICP-OES: The estimation and evaluation of heavy metals in smokers and RT-PCR patient's negative samples was carried out by using an analytical technique ICP-OES.

Analysis of H1N1, HIV, HBV and HCV: ICP-OES were used in order to estimate the heavy metals in different blood of some RT-PCR negative sample of H1N1, HIV, HBV and HCV respectively. The research work shows the estimated results for the level of concentrations of heavy metals (As, B, Pb, Sr and Zn) in all samples were higher than normal (Table 2).

Table 2: Concentration of (As, B, Pb, Sr, Zn) in Negative RT-PCR samples of H1N1, HIV, HBV and HCV

Mean (± SE)	As (µg/dl)	B (µg/dl)	Pb (µg/dl)	Sr (µg/dl)	Zn (µg/dl)
H1N1	141.99 ± 27.31	417.61 ± 68.88	149.14 ± 21.63	6.21 ± 0.90	353.62 ± 60.43
HIV	12.48 ± 0.55	169.83 ± 20.32	138.21 ± 20.17	8.12 ± 0.73	421.45 ± 23.13
HBV	122.35 ± 10.82	160.71 ± 11.79	49.83 ± 4.24	53.73 ± 3.83	1459.39 ± 90.07
HCV	4.81 ± 0.44	3.72 ± 0.33	123.21 ± 7.69	60.85 ± 5.45	180.24 ± 13.49

Comparison of metals with immunity against viral infections: On a subset of samples which were negative on H1N1, HCV, HbsAg and HIV real time PCR, a correlation analysis was done. Results indicate there is a significant association between metals (As, B, Pb, Sr and Zn) and immunity against all these viral infections (P=≤0.05) except HCV. Only As shows positive correlation against HCV infection other metal have no correlation against HCV infection.

DISCUSSION

This study was aimed to find the levels of trace metals in RT PCR negative samples of various viral infections and further assess the correlation of these metals with the immune status of the patients. This study highlights the importance of trace metals in the innate immune system and host resistance to infection. Negative PCR samples were used in this study because of the presence of antibodies against virus in ELISA. In the present study all 67 samples were positive by ICT and ELISA techniques, however only 25 samples were found to be negative by RT PCR technique which indicate that PCR should be the choice of diagnosis for viral infection rather than ICT or ELISA technique[15].

A similar study reported higher levels of trace elements in patients and suggest that method exposure increase the inflammatory response to influenza, and this is in agreement with our results showing higher levels of As in blood and showing a significant relationship with immunity against HBV, HCV, HIV and H1N1 infections[16]. Zn influences the function of immune regulators used in the innate and adaptive immune response, our results indicate zinc is compulsory for our immune system and provides protection against viral infections.

It has been accepted widely that both the acute or chronic exposure to Cd, As or Pb are related with the modulation of immune system[17]. Our results showed that arsenic play an important role against viral infections. Boron is a center of attention on biochemical pathways and biomolecular applicable to immune feature and our results also show that boron plays an important function in immunity against viral infections[18]. There is enough evidence that trace elements supplements especially zinc play a significant role in preventing liver cirrhosis and Zn supplementations are used widely as an adjunct therapy against HIV infection is used widely[19,20].

Another cross-sectional study done in USA assesses the correlation between As levels and the seroprevalence of hepatitis B (HBV) infection and shows a positive relation between As present in blood and immunity against Hepatitis B infections [21]. Another study showed similar results to our study by showing high levels of Zn show enhanced immunity in HBV negative patients the communication among Zn and the immune machine is complex[22]. Our findings clearly support other studies that if the level of zinc in the body is affected it also affects the immune system. Our study also indicates there is a positive correlation between immunity against viral infections and level of trace elements. Another

study reports similar results showing significant correlation between level of trace elements and HCV infection [23,24,25].

CONCLUSION

This study report high levels of As, B, Pb, Sr, and Zn in all the RT-PCR negative samples for H1N1, HIV, HCV and HBV and all of these metals has shown to have a positive correlation with immunity against HBV, H1N1 and HIV infections except HCV infection which is only correlated with As. Therefore, it can be concluded trace elements are highly important for having a good immune response against viral infections and trace elements should be monitored and maintained in every individual in other to boost a normal immune response against viral infections.

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