Alterations in immunoglobulins and cytokine levels in blood of malathion poisoning cases

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The excessive exposure and use of malathion, an organophosphate pesticide, has lead to deleterious effects on human health. Chronic exposure to organophosphates has been shown to suppress immune system in experimental animals. Therefore, in this study, we have investigated the immunoglobulins (IgG, IgM, IgE and IgA) and cytokines (IL-2, IL-4, IFN-γ and TNF-α) levels in blood of malathion poisoning cases, admitted in Guru Teg Bahadur Hospital (University of Delhi), Dilshad Garden, Delhi, India. All the seriously ill patients of malathion poisoning showed significant levels of residue (503-702 mg/L). While no significant changes were found in Ig levels in blood of malathion poisoning cases, there was a significant increase in IL-2, IL-4 and TNF-α levels in blood of malathion poisoning cases, and significant decrease in IFN-γ level, as compared to normal subjects. This study demonstrated altered levels of cytokines and interleukins in serum in response to malathion exposure.

Keywords: Malathion, Cytokines, Interleukins, Immunoglobulins, Pesticides

The widespread use of pesticides in public health programmes and agriculture has caused severe environmental problems and potential health hazards. Malathion (O,O-dimethyl S-1-2 bis-ethoxycarbonyl ethyl phosphorodithioate) is a widely used organophosphate and has extreme stability and slow metabolism. Excessive use and exposure of malathion has also led to environmental contamination and health hazard1,2. Many of the pesticides, including organophosphates are known to cause impairment or suppression of immune system3-4. Recent reports focus attention on oxidative stress induced immunotoxicity5. Evidences have shown that pesticides may target the function of cellular, subcellular or molecular components of the immune system6.

Earlier study from our laboratory has shown marked immunological effects of malathion exposure in different experimental animals7. Malathion exposure results in a decrease in both cellular as well as humoral immune responses in experimental animals. However, there is a paucity of information in the literature regarding the immunotoxicity of malathion in humans. To our knowledge, no reports are available in relation to cytokines and immunoglobulin (Ig) levels in malathion poisoning cases. Cytokines function as specific chemical mediators that ultimately regulate growth and differentiation of cells8. Therefore, in this study, we have investigated the Ig levels (IgM, IgE, IgD and IgA) and immunoregulatory cytokines/interleukins (IL-2, IL-4, TNF-α and IFN-γ) in blood of malathion poisoning cases.

Materials and Methods

The study group consisted of 35 patients of malathion poisoning cases, irrespective of age and sex, admitted in Guru Teg Bahadur Hospital, Delhi, India, during the period 2000-2002. The age of suspected poisoning cases ranged between 20-35 yrs for males and 16-28 yrs for females. The subjects were diagnosed for malathion poisoning by clinical characteristics, manifestations, history, presence of specific pesticide residue and acetylcholine esterase (AChE) activity in erythrocytes and were treated symptomatically with an acetyl choline inhibitor (atropine) if required. Patients with active infection or any clinically detectable illness or with history of occupational exposure to pesticides were excluded from the study. Twenty-five age and sex-matched healthy volunteers with no history of pesticide exposure or consumption of alcohol, drugs like warfarin, phenobarbitone etc. or any immunomodulatory drugs, and not suffering from any clinically detectable illness constituted the control group. An informed consent was obtained from all the participants and the study had the approval of the Institutional Ethical Committee.

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Blood samples collected within 24 h were used for estimation of pesticide residue levels by HPLC and AChE activity as described earlier, and samples collected within 10 days were used for immunological assays. Blood was also sampled within 24 h, 48 h, 72 h, one week, 10th day, 14th day, 21 days and 30 days (depending upon the severity of poisoning) after hospital admission for follow-up of clinical characteristics and AChE activity estimation. All serum samples were stored at -20°C until analysis. Serum Igs levels were measured by a radial immunodiffusion technique. Cytokine (IL-2, IL-4, TNF-α, and IFN-γ) levels were measured in serum samples using ELISA kits obtained from Diaclone Research, France.

Results and Discussion

All the seriously ill patients admitted after suspected pesticide poisoning were treated symptomatically and investigated for various routine biochemical and hematological profile. Elevated levels of the pesticide residue in the range of 382–500 mg/L blood were detected on the 1st day of hospitalization. Significant decrease was observed in AChE activity in RBC of malathion poisoning cases (8.26 ± 2.1 KAU/L), in comparison to control subjects (10.43 ± 1.9 KAU/L). Effect of malathion on immunoglobulins (IgM, IgG, IgA, IgE) and cytokine (IL-2, IL-4, TNF-α and IFN-γ) levels in serum is presented in Table 1. No significant change was found in Igs levels, whereas a significant increase in IL-2, IL-4 and TNF-α levels was observed in malathion poisoning cases, while IFN-γ level decreased significantly as compared to normal subjects.

Experimental and clinical studies indicate that long-term low exposure to organophosphates may induce significant changes in neurobehavioural patterns which is a potential health hazard. Like all other organophosphate pesticides, malathion may influence inhibition of AChE, neuropathy, physiological and pathological conditions and also act directly or indirectly on lymphoid cell distribution, Ig metabolism, T or B cell functions, macrophages cooperation and macromolecular biosynthesis. Immune suppression after malathion exposure is possible as organophosphates bind to esterase, a vital membrane bound protein that helps immune cells to interact with and destroy foreign organisms. A correlation between AChE suppression and immune responses by organophosphates has also been suggested. The decreased activity of AChE after malathion exposure inhibits the E-rosetting of T-lymphocytes. Moreover, inhibition of AChE results in an accumulation of the amino acid neurotransmitter acetylcholine (ACh), which stimulates lymphocytes, elevates concentrations of cellular c-GMP and increases the motility and cytotoxicity of lymphocytes.

A significant decrease in serum IFN-γ and increase in IL-2, IL-4, and TNF-α levels in malathion poisoning cases in present study suggests interaction of malathion with the early events of lymphocyte activation. Cytokines are the cellular mediators of the immune system, synthesized by lymphoid cells or nonlymphoid cells and bind to specific receptors on target cells. Also, many cytokines inhibit or induce production and function of other cytokines. Therefore, natural regulation of cytokines network becomes increasingly important.

The helper CD4 cells, which recognize antigen-presenting cells, initiate a proliferative response with a restricted cytokine secretion pattern of Th1, Th2 or Th0 cells. The Th1 cytokines (IL-2 and IFN-γ) play an important role in cell mediated immunity (CMI) and chronic inflammation. CD4 cells regulate humoral immune response and B cells produce Igs, so the balance between a largely cellular or a humoral response is orchestrated by cytokines derived from CD4 Th cells. Macrophages, Th1 cells, T lymphocytes, parenchymal cells and leucocytes in pathophysiological situation secrete TNF-α. Not only it promotes inflammatory and cellular responses but also provides protection against intracellular pathogens. In contrast, IL-4, which promotes humoral

### Table 1—Effect of malathion on immunoglobulins and cytokines levels in blood
[Values represent absolute mean of serum cytokine concentration (pg/ml) ± SD]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Malathion poisoning Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunoglobulins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM (IU/ L)</td>
<td>1.16 ± 0.14</td>
<td>0.93 ± 0.31</td>
</tr>
<tr>
<td>IgG (IU/ L)</td>
<td>14.57 ± 2.11</td>
<td>12.95 ± 2.79</td>
</tr>
<tr>
<td>IgA (IU/ L)</td>
<td>1.86 ± 0.11</td>
<td>1.65 ± 0.20</td>
</tr>
<tr>
<td>IgE (IU/ L)</td>
<td>0.118 ± 0.019</td>
<td>0.105 ± 0.019</td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2 (pg/ml)</td>
<td>16.3 ± 2.39</td>
<td>57.69 ± 3.9*</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>0.78 ± 0.09</td>
<td>1.76 ± 0.49*</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>7.25 ± 0.82</td>
<td>29.17 ± 6.46*</td>
</tr>
<tr>
<td>IFN-γ (pg/ml)</td>
<td>2.96 ± 0.57</td>
<td>1.21 ± 0.30*</td>
</tr>
</tbody>
</table>

* Significantly different from normal subjects (p< 0.05)
immunity is required for elimination of extracellular pathogens by induction of B cell growth, which leads to generation of Igs and reduce the risk of Th1 mediated autoimmunity. Hence, balance between cell-mediated and humoral immunity is required for an effective immunity against infection.

The present study indicates that malathion modulates immune reaction via different mechanisms. Th1-like response is enhanced with release of cytokines (IL-2 and TNF-α) that induce B cell maturation. Th2 response is partially inhibited with cytokines (IL-2 and TNF-α) production. The increase in TNF-α may result from a mechanism to compensate for the decrease in INF-γ production after pesticide exposure and/or loss of NK cell activity. The decrease in IFN-γ level facilitates migration of leucocytes into CNS by enhanced adhesion between leucocytes and endothelial cells. IFN-γ is also known to induce a number of genes, transcription factors and inflammatory mediators\(^1,2,17,20\).

Accumulation of ACh in lymphocytes which leads to mortality and cytotoxicity after malathion exposure suggests reduction in the IFN-γ levels in serum. The role of cytokine in malathion-induced reactions of immune system and possible cytokine interactions are not known. Th2-derived IL-4 stimulates B cell activation and Ig secretion, and Th1-derived IFN-γ causes antagonistic response. The increased IL-2 and IL-4 levels in the present study may be due to proliferation of T cells. The decrease in IFN-γ may be caused by inward lymphocyte mortality and cytotoxicity after malathion exposure.

The present study demonstrates that serum levels of certain cytokines may increase/decrease in response to malathion. This effect may be free radicals-mediated as several studies suggest that cytokines are at times released during oxidative stress\(^1,20\). As the Th2-derived IL-4 stimulates B-cell activation as well as Igs secretion and the Th1 derived IFN-γ causes antagonistic responses, these cytokines may be suitable markers for immunotoxic effects of malathion. Hence, serum IL-4 and IFN-γ measurements may provide an easier method with higher sensitivity for demonstration of immunotoxicological potency of malathion with respect to the functional ability of lymphatic system.

In conclusion, the present study demonstrates that malathion poisoning may influence the immune system in humans through cytokines or suppressing the lymphocytes functions via cholinergic stimulation.

Acknowledgement

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References

10. ATSDR (2005) Agency for Toxic Substances and Diseases Registry, 1-10