An Epidemiologic Study to Screen for Chronic Myelocytic Leukemia in War Victims Exposed to Mustard Gas

Mostafa Ghanei[1] and Ali Akbar Vosoghi[2]

[1]Chemical Warfare Victims Research Department, Baqiyatallah University of Medical Sciences, Tehran, Iran; [2]Chemical Warfare Victims Department, Isfahan University of Medical Sciences, Isfahan, Iran

Chemical agents such as mustard gas (or sulfur mustard), which has alkylating characteristics, were used against Iranian combatants in the Iraq–Iran war. Previous studies have not shown a strong link between these chemical agents and the development of chronic myelocytic leukemia (CML). The purpose of this study was to evaluate the increased risk of CML development in Iranian soldiers exposed to mustard gas during the war. Based on a descriptive study of 2,500 cases with documented exposure to various chemical warfare agents, 665 patients had documented exposure to mustard gas. We screened the latter using the leukocyte alkaline phosphatase (LAP) test and performed further cytochemical studies on cases with positive results. From among the 665 cases with documented exposure to mustard gas, 9 cases had LAP scores < 20; 2 of these 9 cases had CML and a score of zero (0.3%). We detected cytogenetic abnormalities in 7 patients with low LAP scores and atypical lymphocytes of 5–11% in 40 patients. The risk ratio of CML developing in victims exposed to mustard gas (cutaneous or respiratory) may be higher in comparison with the normal population, although confounding factors (e.g., the possibility of exposure to combined chemical agents, excluding patients who did not manifest blisters) limited our results. Because the increased development of CML in young patients with a documented history of exposure to mustard gas cannot be disregarded, further studies are needed. *Key words*: chronic myelocytic leukemia, Iran, mustard gas, war victims. *Environ Health Perspect* 110:519–521 (2002). [Online 3 April 2002]

http://ehpnet1.niehs.nih.gov/docs/2002/110p519-521ghanei/abstract.html

Thousands of Iranian soldiers were exposed to mustard gas during the Iraq-Iran war from 1983 to 1989 (*1*). Mustard gas, also known as sulfur mustard (SM), is an alkylating agent with mutagenic effects (*2*). Mustard gas became recognized as the first of a new class of toxins known as "blistering agents." Skin contact, which could occur via airborne concentrations that penetrate easily through most clothing, led to damage ranging from a sunburnlike scald to bullous necrosis. Eye contact with SM caused severe corneal injury, and inhalation induced acute tracheobronchitis with massive epithelial sloughing (*3*).

Previous studies have shown that the risk of lung cancer increases after long-term, low-level exposure to mustard gas (*4*). There have been no reports of chronic myelocytic leukemia (CML) following SM exposure or of a confirmed relationship between alkylating agents and CML (*5*). According to data from one of the military health centers, two cases of CML have been reported in 2 consecutive years in soldiers who had a history of mustard gas exposure during the war. We therefore undertook this study to seek a relationship between SM exposure and the development of CML.

Materials and Methods

Among 2,500 chemical warfare victims in Isfahan province, Iran, we studied 665 war victims exposed to SM during the Iraq–Iran war. SM poisoning was documented in this population by military health services within a few hours of injury. Military specialists documented the type of chemical agent and reported to health center services within a few hours of attacks.

Inclusion criteria. All included cases had documented SM exposure. These victims all developed blisters to areas of their bodies within a few hours of exposure, they had transient decreased vision lasting several days, and they had respiratory symptoms such as chronic bronchitis for several years.

Exclusion criteria. We excluded patients with fever, active infectious disease, or a history of hormone use [i.e., adrenocorticotropic hormone (ACTH) or adrenal 17-OH-corticosteroids]. We completed a demographic questionnaire for each patient. Complete blood count (CBC), peripheral blood smear, and bone marrow smears were performed and studied by a hematologist. Cytochemical staining of peripheral blood smears for leukocyte alkaline phosphatase (LAP) tests was performed using the Kaplow method (*6*). Smears were studied immediately after staining, and 100 neutrophil granules were examined. To score neutrophils on a blood smear, 100 consecutive band neutrophils and polymorphonuclear leukocytes were counted and rated by intensity from 0 to 4+, deriving a final LAP score of 0–200. We considered scores > 20 normal and < 20 abnormal; abnormal results were studied with complementary tests. To examine the value of LAP activity, we referred single cells to one of three groups: negative (0 score), weakly positive (+1, +2 score), and strongly positive (+3, +4 score). We performed cytogenetic studies on bone marrow aspiration samples of low–LAP-score cases after standard culture in cases with LAP activity < 20. Cytogenetic readers did not know anything about the cases or their data. We measured serum zinc by an atomic absorption method and performed statistical analysis using the Student's *t*-test to compare means.

Control group. We selected healthy men of the same age from a premarriage counseling center to use as controls. We performed only the CBC test in this group.

Results

The mean ages (± SD) were 34 ± 3 years for the patients and 34 ± 1.2 for the control group. The average time lapse from injury was 12 ± 2 years. Table 1 indicates the means of the blood indices of the two groups, along with the statistical results. The mean LAP score of the patients was 59.9 ± 18.2. Nine cases had LAP scores < 20, and two cases had a score of 0 and were reported as CML cases; the other data for these two cases are shown as cases 1 and 2 in Tables 2 and 3. The CBCs with differentiation, Philadelphia chromosome, and serum zinc level for these patients are listed in Table 2. In case 1, the Philadelphia chromosome was positive with typical laboratory manifestations of CML, the LAP score was 0, peripheral blood smears had high white blood cell counts and a shift to the left compatible with CML. Case 2 also showed cellular morphology of peripheral blood smears and bone marrow typical of CML and low serum zinc but was negative for the Philadelphia chromosome. The cytogenetic findings of these two cases and others with low LAP scores are shown in Table 3. From among the nine cases affected by SM, we found seven cases (77%) of karyotypic clones that were diploid and two cases (23%) that were pseudodiploid, including one with

Address correspondence to M. Ghanei, Research Department, Baqiyatallah University of Medical Sciences, Vanak Square Mollasadra Avenue, 19945-587 Tehran, Iran. Telephone: 98-218053770. Fax: 98-218040106. E-mail: m.ghanei@bmsu.ac.ir

We express our sincere appreciation to J. Goldman for his help in preparation of the manuscript and to H. Abolghasem, A. Saadat, and P. Mahdipour for their help in the hematology and cytogenic studies.

Received 23 July 2001; accepted 23 November 2001.

the classic Philadelphia chromosome. In all of nine cases, the other minor clones (Table 3) accompanied the major clonal abnormalities. Three of these had hyperdiploid clones.

Discussion and Conclusion

Acute complications of SM on the hematopoietic system have been reported repeatedly during the Iraq–Iran war (*7–9*). Aplastic anemia resulting in death, as well as reversible patterns, has also been documented (*8,10*). We have found no published data documenting CML development after exposure to SM. In the cases in our study, SM exposure was documented because of blisters on the skin and long-term respiratory disorders, symptoms caused by no other chemical warfare agent. In our study group, blood indices revealed significant differences with the control group because of the large sample, but this was not meaningful clinically. Although victims with severe respiratory exposure to SM do not survive, victims do survive when exposure is not excessive. Patients with fever, active infectious disease, or a history of drug use (i.e., ACTH, adrenal 17-OH-corticosteroids) were excluded from this study because these factors have been shown to affect the LAP test (*11*).

CML is a disease of the elderly, and its peak incidence is usually in the fifth and sixth decades of life. The annual incidence of CML is about 1 per 100,000, and it appears to be constant worldwide. From among 2,500 cases with documented exposure to various chemical warfare agents, 665 patients had documented exposure to mustard gas. We found two cases with CML in 2 consecutive years. Although we could not ascertain whether combined chemical gases were used along with SM in the study group, we verified exposure to this gas in all cases by the aforementioned inclusion criteria. In our study, we chose the control group from among the normal population and not from war veterans because low-dose exposure without significant symptoms may have been present among these soldiers. If we accept only the case with positive Philadelphia chromosome as a CML case, then this may be a sporadic case with no relation to mustard gas exposure. However, if we consider it as an outcome of SM, then the occurrence rate is 1 case per 2,500, and this prevalence is 400 times greater than that in the normal population. These findings compelled us to evaluate all of SM-exposed victims for leukemia in a well-designed study that should be completed by 2004.

LAP scoring is a method that is widely used in diagnostic hematology. This method establishes a discriminating factor between CML and other conditions with leukemoid reactions (*12,13*). LAP is consistently reduced in persons with CML, paroxysmal nocturnal

Table 1. Blood indices (mean ± SD) and statistical analysis comparing patient and control groups.

	Control	Patients	*p*-Value
White blood cells	6.91 ± 1.87	6.67 ± 4.36	0.214
Red blood cells	5.56 ± 0.67	5.46 ± 0.49	0.006
Hemoglobin	15.39 ± 1.76	15.60 ± 1.21	0.03
Hematocrit	47.07 ± 4.60	47.91 ± 3.43	0.001
Mean corpuscular hemoglobin	27.8 ± 2.39	28.72 ± 2.25	< 0.001
Mean cell hemoglobin concentration	32.66 ± 1.66	32.59 ± 1.09	0.45
Mean cell volume	85.17 ± 6.18	87.63 ± 8.10	< 0.001
Platelets	226.80 ± 52.42	208.44 ± 66.6	< 0.001
Neutrophils	4.09 ± 1.56	3.60 ± 1.39	< 0.001
Lymphocytes	1.99 ± 0.61	2.05 ± 0.63	0.08
Monocytes	0.47 ± 0.15	0.43 ± 0.19	< 0.001
Eosinophils	0.19 ± 0.17	0.23 ± 0.23	< 0.001
Basophils	0.05 ± 0.03	0.11 ± 0.36	< 0.001
Large unstained cells (%)	0.17 ± 0.06	0.17 ± 0.09	0.09

Table 2. Differential leukocyte count and differentiation with Philadelphia chromosome and zinc levels in nine male patients.

Case	Year of exposure	Age (years)	Zinc (mg/dL)	Philadelphia chromosome	NRBC (%)	Immature (%)	Band (%)	mon (%)	bas (%)	eos (%)	lym (%)	PMN (%)	WBC (n/mm^3)[a]
1	1988	42	73	+	1	6	5	4	0	0	25	60	28.1
2	1988	31	50	–	1	7	24	3	0	1	8	56	54.8
3	1987	32	75	–	0	0	0	1	0	1	43	55	7.5
4	1985	45	72	–	0	0	0	1	0	6	41	52	7.6
5	1985	42	69	–	0	0	0	2	0	0	38	60	5.8
6	1985	34	77	–	0	0	0	1	0	1	27	71	6.5
7	1983	37	87	–	0	0	0	3	0	1	32	64	5.9
8	1984	36	78	–	0	0	0	2	0	0	40	58	6.5
9	1988	30	81	–	0	0	0	1	0	0	44	55	5.1

Abbreviations: Band, band neutrophils; bas, basophils; eos, eosinophils; lym, lymphocytes; mon, monocytes; NRBC, normal red blood cells; PMN, polymorphonuclear leukocytes; WBC, white blood cells.
[a]× 1,000.

Table 3. Cytogenetic findings and distribution of karyotypic cell lines in nine male patients.

Case	Hyperdiploidy	Pseudodiploidy	Mitoses with chromatid breaks	Diploidy	Diagnosis	Age (years)
1	—	46/XY/ t(9.22)(q34/q11) [13]	—	46/XY [5]	CML	42
2	46/XY/including 2 fragments [2] 47/XY/+21 [4]	46,XY,22q- [4]	3q/6q/14q [6]	46/XY [12]	CML	31
3	—	46/XY 22q- [7] 46/XY/-1/+ marker/22q- [1]	—	46/XY [12]	Healthy	32
4	47/XY/+22 [4]	46/XY/22q- [9]	—	46/XY [10]	Healthy	45
5	—	46/XY/including fragments.[13]	—	46/XY [4]	Healthy	42
6	—	46/XY/-18/+marker [5]	—	46/XY [10]	Healthy	34
7	47/XY/+2 [3] 47/XY/?+4 [1]	—	—	46/XY [12]	Healthy	37
8	—	46/XY/including 2 fragments [2] 46/XY/deletions (2) (pter)	—	46/XY [12]	Healthy	36
9	47,xy,+13 [2] 49,xy,+16,+18,+21 [1]	—	3q,6q,14q and 9 gaps (ch 14) [6]	46/XY [20]	Healthy	29

Abbreviations: ch, chromosome; pter, end of short arm of chromosome. The numbers inside the brackets indicate the number of cells.

hemoglobinuria, and hypophosphatasia, and can be used to diagnose these disorders. Low levels of LAP have also been reported in thrombotic thrombocytic purpura, aplastic anemia, and sickle cell disease. We ruled out these conditions in seven cases without CML by clinical and laboratory review of the findings. Although it is not obvious why LAP scores decreased, this decrease may be related to the presence of other cytogenetic abnormalities that are present in these patients, and follow-up studies should determine that. Previous studies have shown that the LAP test is a first-line test for CML and generally precedes a diagnostic algorithm that also includes bone marrow biopsy, cytogenetic analysis, and molecular diagnostics (*14*). Although *bcr/abl* would have helped to definitively confirm CML in our cases, we did not find a laboratory expert in this analysis in Iran at the time of our study. The Philadelphia translocation results in the fusion of the *bcr* and *abl* genes in a head-to-tail fashion. The *bcr* gene loses a 3′ section, which is replaced by a 3′ *abl* sequence from chromosome 9.

This study also shows that the LAP test can be used as a screening test for detection of CML in high-risk patients. Previous reports have shown that ionizing irradiation (*15*) and occupational exposure to nonionizing radiation through electrical work are linked particularly to CML (*16*). Benzene exposure is also acknowledged to increase the risk of myeloid leukemia (*17*) and case reports have linked CML with ingestion of cytotoxic drugs (*18*). Because of failure to follow up thousands of World War II veterans (in Australia, the United States, and Canada) exposed to SM, there is no report regarding CML development. To our knowledge, this is the first case report that links inhalation of SM, an alkylating agent, with the development of CML. Our findings indicate that the incidence of CML among war veterans is significantly greater than in the general population.

References and Notes

1. United Nations Security Council. Report of the Specialist Appointed by the Secretary General to Investigate Allegation by the Islamic Republic of Iran Concerning the use of Chemical Weapons. Report no. 3/16433. New York:United Nations Security Council, 1984.
2. Ludlum DB, Papirmeister B. DNA modification by sulfur mustards and nitrosoureas and repairs of these lesions. Basic Life Sci 38:119–125 (1986).
3. Medical Research Committee. An Atlas of Gas Poisoning. London:Medical Research Council, 1918.
4. Tokuoka S, Hayashi Y, Inai K, Egawa H, Aoki Y, Akamizu H, Eto R, Nishida T, Ohe K, Kobuke T, et al. Early cancer and related lesions in the bronchial epithelium in former workers of mustard gas factory. Acta Pathol Jpn 36:533–342 (1986)
5. Hughes TP, Goldman JM. Chronic myeloid leukemia. In: Hematology: Basic Principles and Practice (Hoffman R, ed). New York:Churchill Livingstone, 1991;854.
6. Kaplow LS. A histochemical procedure for localizing and evaluating leukocyte alkaline phosphatase activity in smears of blood and marrow. Blood 10:1023–1029 (1955).
7. Moradi A, Sodeifi B, Abdollahi A. Clinical presentation of chemical warfare injuries. Iran J Med Sci 13:1–5 (1986).
8. Tabarestani M, Farhoudi M, Balali M. Stem cell and erythroid precursors disorders in three patients with sulfur mustard poisoning. In: Proceedings of the First International Medical Congress on Chemical Warfare Agents in Iran, June 1988, Mashhad University of Medical Sciences, Mashhad, Iran. Mashhad, Iran:Mashhad University Press, 1988;F10.
9. Tabarestani M, Balali M, Farhoudi M. Hematologic findings of sulphur mustard poisoning in Iranian combatants. Med J Islamic Rep of Iran 3:185–189 (1990).
10. Kumar P, Tabei S, Sotoodeh M. Aplastic anemia in chemical war injury patients. In: Proceedings of the First International Medical Congress on Chemical Warfare Agents in Iran, June 1988, Mashhad University of Medical Sciences, Mashhad, Iran. Mashhad, Iran:Mashhad University Press, 1988;78–79.
11. Valentine WN, Follette JH, Solomon DH, Reynolds J. The relationship of leukocyte alkaline phosphatase to "stress" to ACTH, and to adrenal 17-OH-corticosteroids. J Lab Clin Med 49:723–737 (1957).
12. Wachstein M. Alkaline phosphatase activity in normal and abnormal human blood and bone marrow cells. J Lab Chin Med 31:1–17 (1946).
13. Okun DB, Tanaka KR. Leukocyte alkaline phosphatase. Am J Hematol 4:293–299 (1978).
14. Depalma L, Delgado P, Werener M. Diagnostic discrimination and cost effective assay strategy for leukocyte alkaline phosphatase. Clin Chim Acta15:244:83–90 (1996).
15. Gunz FW. The epidemiology and genetics of the chronic leukemias. Clin Haematol 6:3–20 (1977).
16. Milham S. Silent keys: leukemia mortality in amateur radio operators [Letter]. Lancet i:812 (1985).
17. M. Malignancies due to occupational exposure to benzene. Am J Ind Med 7:395–402 (1985).
18. Wanders J, Wattendorff AR, Endtz LJ, den Nijs JJ, Leeksma CH. Chronic myeloid leukemia in myesthenia gravis after long-term treatment with 6-mercaptopurine. Acta Med Scand 210:235–238 (1981).