Chronic lymphocytic leukaemia in Egyptian farm workers exposed to pesticides

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الابيضاض اللمفاوي المزمن في عمال المزارع المصريين الذين يتعرضون لمبيدات الآفات

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خلاصة: كان هدف هذه الدراسة تبيان العلاقة بين حدوث الابيضاض اللمفاوي المزمن في عمال المزارع وبين تعرضهم لمبيدات الآفات. فأخذت عينات دم من 932 من عمال المزارع المصريين الذكور، الذين يتعرضون لمبيدات الآفات. كما أخذت عينات دم من مجموعة شاهدة تتكون من 932 شخصاً من الذكور، من نفس العمر والحالة الاجتماعية الاقتصادية، ممن لا يعملون بالزراعة ولا يتعرضون عادةً لمبيدات الآفات. وتبيَّن أن عمال المزارع كانت لديهم تعدادات مرتفعة بدرجة ملحوظة من اللمفاويات وكريات الدم البيضاء والصفيحات. ووجدت خلايا غير ناضجة لدى 5% تقريباً من عمال المزارع الذين تزيد أعمارهم عن أربعين سنة. ووجد الابيضاض اللمفاوي المزمن لدى اثنين من عمال المزارع بينما لم يوجد لدى أي فرد من المجموعة الشاهدة، الأمر الذي يكشف عن خطر نسبي غير محدَّد، وخطر مسبب بنسبة 2.1 في الألف، وهي نسبة لا يعتد بها إحصائياً.

ABSTRACT We aimed to explore the relationship between chronic lymphocytic leukaemia and pesticide exposure among farm workers. Blood samples were obtained from 932 male Egyptian farm workers exposed to pesticides and from a control group of 932 males of similar age and socioeconomic status who were not involved in farming and did not normally deal with pesticides. The farm workers had significantly higher lymphocyte, white blood corpuscle and platelet counts. About 5% of the farm workers over 40 years had immature cells. Two of the farm workers and none of the control group had chronic lymphocytic leukaemia, giving an undefined relative risk and an attributable risk of 2.1 per 1000, which was not statistically significant.

Leucémie lymphoïde chronique chez des ouvriers agricoles égyptiens exposés aux pesticides

RESUME Notre but était d'étudier la relation entre la leucémie lymphoïde chronique et l'exposition aux pesticides chez des ouvriers agricoles. Des échantillons de sang ont été prélevés chez 932 ouvriers agricoles égyptiens exposés aux pesticides et chez un groupe témoin de 932 hommes d'âge et de condition socio-économique similaires qui n'étaient pas engagés dans des activités agricoles et normalement n'étaient pas en contact avec des pesticides. Chez les ouvriers agricoles, le nombre de lymphocytes, de globules blancs et de plaquettes était significativement plus élevé. Environ 5% des ouvriers agricoles âgés de plus de 40 ans avaient des cellules immatures. Deux des ouvriers agricoles et zéro individu du groupe témoin avaient une leucémie lymphoïde chronique: cela donne un risque relatif non défini et un risque attribuable de 2,1 pour mille, ce qui n'est pas statistiquement significatif.

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Introduction

Chronic lymphocytic leukaemia (CLL) is a neoplasm resulting from the proliferation of a single cell clone that is in an early stage of lymph differentiation and maturation. This phenomenon of arrested maturation is associated with long-lived cells, which leads to lymphocyte accumulation [*1*].

Environmental exposure is known to play a role in the development of acute leukaemias, but the link between environmental exposure and chronic leukaemias, including CLL, is less clear. Several studies have been carried out to examine the relationship between environmental exposure and CLL, but while some showed a positive correlation between CLL and electromagnetic radiation, benzene and asbestos exposure, others showed none. Most of these studies were based upon retrospective examination of epidemiological data, including death certificates and cancer registration and the contradictions may be due to differing methods for assuming the nature of environmental hazards in relation to the type of work of the patients in the cases studied [*2–8*].

Some studies have found an abnormally high incidence of CLL among farmers in the United States of America, although these findings have not been confirmed in Scandinavian studies [*3*]. Two case–control studies on CLL and non-Hodgkin lymphomas (NHLs) in farm-animal breeding workers in an agricultural area in Italy found that these workers were at a high risk for CLL and low-grade NHLs. Although one study found that this could be due to exposure to animal-transmitted diseases, specific chemicals used in breeding, or the use of agricultural chemicals [*9*], the other ascribed it specifically to insecticides, including carbamates, phosphates and DDT [*10*].

This study aimed to find any relationship between CLL and exposure to insecticides in farm workers. Because of the variable results obtained in case–control studies and the lack of registration of CLL in Egypt, this study was based on a field survey.

Subjects and methods

A population of 932 males from the governorates of Alexandria, Beheira and Marsa Matruh were selected for investigation. All worked in farming and had been exposed to pesticides, mainly cholinesterase inhibitors and, less commonly, halogenated insecticides, for 3 to 42 years. All of the participants were covered by health insurance and had been given periodic medical examinations. The mean age of the exposed population ± standard deviation was 44.56 ± 9.36 years. A further 932 males not working in farming, not dealing with pesticides and of similar socioeconomic status were selected as a control population. Their mean age was 43.20 ± 10.10 years.

A blood sample was drawn from each individual from both the exposed and control populations. Acetyl cholinesterase enzyme (AChE) activity was measured immediately after taking the blood sample using a spectrophotometer at wavelength 412 nm [*11*] and red blood corpuscles were counted microscopically. Peripheral blood for each individual was examined to determine haemoglobin (g/dL), white blood corpuscle count ($\times 10^9$/L), absolute lymphocyte count ($\times 10^9$/L) and platelet count ($\times 10^9$/L). Cases were diagnosed as CLL when sustained lymphocytosis was above 10×10^9/L in peripheral blood [*12*]. Such cases were referred for bone marrow examination.

Statistical analysis

The data were analysed using *SPSS* version 7.5. $P < 0.05$ was used to indicate statistical significance. The arithmetic mean, standard deviation and proportion were used as summary statistics and the chi-squared test was used for comparison among proportions [*13*]. The Kolmogorov–Smirnov one-sample test was used to explore the distribution of quantitative variables. Where there was deviation from the normal distribution, the Wilcoxon–Mann–Whitney test was used for comparison of two independent groups; otherwise the variable was logarithmically transformed and the parametric test was used [*14*]. The attributable risk for CLL was computed to estimate the proportion of risk that could be avoided by eliminating exposure to pesticides [*15*]. Stepwise discriminant analysis was used to explore the best linear combination of variables that could classify participants into one of two groups: those having immature leukocytic cells and those without. The efficiency of the model is indicated by a minimum Wilk's lambda, a maximum canonical correlation, a significant model chi-squared, and a maximum percentage of correct classifications [*16*].

Results

The majority of both the exposed (72.3%) and control (66.0%) populations were over 40 years of age. A significantly higher per-

Table 1 Characteristics of exposed and control groups

Variable	Exposed group (n = 930)[a] No.	%	Control group (n = 932) No.	%	Test of signicance
Age (years)					
≤ 40	258	27.7	317	34.0	$\chi^2_1 = 8.58^b$
> 40	672	72.3	615	66.0	
Haemoglobin (g/dL)					
< 10	9	1.0	6	0.6	$\chi^2_3 = 18.10^c$
10–	126	13.5	142	15.2	
12–	730	78.5	669	71.8	
14–15	65	7.0	115	12.3	
Lymphocytes (× 10⁹/L)					
< 4	814	87.5	891	95.6	$\chi^2_2 = 43.50^c$
4–	102	11.0	41	4.4	
5+	14	1.5	0	0.0	
Platelets (× 10⁹/L)					
< 250	919	98.8	930	99.8	$\chi^2_1 = 6.29^d$
250+	11	1.2	2	0.2	
White blood corpuscles (× 10⁹/L)					
4–	840	90.3	880	94.4	$\chi^2_1 = 11.10^b$
7+	90	9.7	52	5.6	

[a]*Excluding two members of the exposed group with CLL* [b]*Statistically significant (P < 0.01)*
[c]*Statistically significant (P < 0.001)* [d]*Statistically significant (P < 0.05)*

centage of the exposed group had lymphocyte counts of ≥ 4 × 10^9/L (χ^2_2 = 43.50, $P < 0.001$) and haemoglobin levels were found to be significantly lower among the farming group as well (χ^2_3 = 18.10, $P < 0.001$). The exposed participants also showed significantly higher white blood corpuscle and platelet counts (χ^2_1 = 11.10, $P < 0.01$ and χ^2_1 = 6.29, $P < 0.05$ respectively) (Table 1).

It has been reported in previous studies that CLL occurs predominantly in older persons — 90% of patients are over 50 years of age and two-thirds are over 60 years [*17*]. Accordingly, the participants were stratified by age. Those in the exposed group who were over 40 years were found to differ significantly from the corresponding control group in terms of AChE activity level, haemoglobin level, platelet count, white blood corpuscle count and absolute lymphocyte count. In the 40 years and younger range, the exposed participants differed significantly from the controls in acetyl cholinesterase activity level, white blood corpuscle count and absolute lymphocyte count (Table 2).

Two of the exposed participants and none of the control group were found to suffer from CLL. The mean age of the two participants with CLL ± standard deviation was 56.0 ± 2.83 years and they had been working for a mean of 25.50 ± 3.54 years compared to 19.16 ± 9.61 years for the remainder of the exposed group. Their average AChE activity level was significantly lower (73.00% ± 1.41%) than the other exposed participants (82.92% ± 5.61%). The relative risk of CLL in relation to exposure was undefined and the attributable risk was 2.1 per 1000 (95% confidence interval –0.80 to 5.1).

Table 2 Characteristics of exposed and control groups by age

Variable	Age (years) ≤ 40 Exposed (*n* = 258)	≤ 40 Control (*n* = 317)	> 40 Exposed (*n* = 672)[a]	> 40 Control (*n* = 615)	Total Exposed (*n* = 930)[a]	Total Control (*n* = 932)
Duration of work (years)	8.54 ± 8.53		23.23 ± 6.32		19.16 ± 9.61	
Haemoglobin (g/dL)	12.40 ± 0.89	12.54 ± 1.20	12.62 ± 5.06[b]	12.32 ± 0.91	12.56 ± 4.33	12.40 ± 1.02
Lymphocytes (× 10^9/L)	2.92 ± 0.65[c]	2.50 ± 0.60	3.02 ± 0.66[c]	2.43 ± 0.50	2.99 ± 0.66[c]	2.45 ± 0.54
Platelets (× 10^9/L)	194.66 ± 22.38	192.20 ± 22.88	199.49 ± 24.61[c]	193.05 ± 22.54	198.15 ± 24.09[c]	192.76 ± 22.65
White blood corpuscles (× 10^9/L)	5.66 ± 0.75[c]	5.37 ± 0.80	5.72 ± 0.91	5.33 ± 0.67	5.74 ± 0.87[c]	5.34 ± 0.71
Acetyl cholinesterase activity (%)	82.64 ± 5.49[c]	98.93 ± 0.58	83.03 ± 5.65[c]	99.21 ± 0.87	82.92 ± 5.61[c]	99.12 ± 0.79

[a]*Excluding two members of the exposed group with CLL* [b]*Statistically significant (P < 0.05)*
[c]*Statistically significant (P < 0.001)* *Values are expressed as mean ± standard deviation.*

Table 3 **Characteristics of the exposed participants over 40 years of age with and without immature cells**

Variable	Immature cells ($n = 34$)	No immature cells ($n = 638$)	Wilcoxon–Mann–Whitney Z
Duration of work (years)	28.62 ± 5.88	22.95 ± 6.21	5.35[a]
Haemoglobin (g/dL)	12.53 ± 0.65	12.62 ± 5.20	1.22
Lymphocytes (× 10^9/L)	3.61 ± 0.67	2.99 ± 0.64	5.48[a]
Platelets (× 10^9/L)	211.91 ± 30.99	198.82 ± 24.06	2.64[b]
White blood corpuscles (× 10^9/L)	7.46 ± 1.13	5.68 ± 0.80	7.46[a]
Acetyl cholinesterase activity (%)	75.65 ± 3.73	83.42 ± 5.47	7.89[a]

[a]Statistically significant (P < 0.001) *[b]Statistically significant (P < 0.01)*
Two members of the exposed group with chronic lymphocytic leukaemia are excluded.
Only two participants with immature cells were under 40 years.
Values are expressed as mean ± standard deviation.

Immature leukocytic cells were found in 36 of the exposed participants, of whom 34 were over 40 years of age. Those with immature leukocytic cells had been working significantly longer than those without immature cells ($Z = 5.35$, $P < 0.001$). AChE activity, platelet count, white blood corpuscle count, and lymphocyte count for the participants with immature cells were found to be significantly different than among participants without immature cells (Table 3). The two cases of CLL had no immature leukocytic cells.

Stepwise discriminant analysis revealed that white blood corpuscle count, AChE activity level, platelet count and duration of work could predict the presence of immature cells in participants, with 91.1% of participants correctly classified and a canonical correlation of 0.51. Those with immature cells were characterized by high white blood corpuscle and platelet counts, longer duration of work and low levels of AChE activity.

Discussion

Workers who have been exposed to organophosphorous pesticides may exhibit haematological changes involving both the quantity and distribution of leukocytes. Low AChE activity levels in association with changes in leukocytes are diagnostic of chronic organophosphorous poisoning [*18*].

The participants in the exposed group showed a variety of haematological changes, including a relative increase in white blood corpuscle and lymphocyte counts and the presence of immature leukocyte corpuscles. Such changes can be attributed to exposure to cholinesterase inhibitor insecticides, and the low AChE activity level found in the exposed participants does indicate exposure to cholinesterase inhibitor insecticides.

The presence of immature leukocytic cells is associated with the relatively high white blood corpuscle and platelet counts. These changes are also associated with a

Table 4 **Stepwise discriminant analysis of variables predicting presence of immature cells among exposed participants over 40 years of age ($n = 672$)**

Variable[a]	Unstandardized CDFC	Standardized CDFC	*F*	Wilk's lambda	Canonical correlation
White blood corpuscles ($\times 10^9$/L)	12.299	0.734	125.03[b]	0.843	0.505
Acetyl cholinesterase activity (%)	–19.702	–0.558	97.41[b]	0.774	
Duration of work (years)	2.917	0.328	73.32[b]	0.752	
Platelets ($\times 10^9$/L)	3.700	0.193	57.03[b]	0.745	
Constant	16.055				

[a]*All variables were logarithmically transformed* [b]*Statistically significant ($P < 0.001$)*
Model $\chi^2_4 = 196.5$, $P < 0.001$ *Percentage correctly classified = 91.1%*
Model sensitivity = 79.4% *Model specificity = 91.7%*
Only two participants with immature cells were under 40 years.
Two members of the exposed group with chronic lymphocytic leukaemia are excluded.
CDFC = canonical discriminant function coefficient

long duration of work in farming and a low level of AChE activity. This association can be taken as a diagnostic criterion of chronic exposure to AChE inhibitors.

Although those having haemoglobin levels of 14–15 g/dL were fewer among those in the exposed group than among the control, there was no apparent characteristic pattern.

The two cases of CLL found among the exposed group did not show a significant relationship to farming. The data indicated an undefined relative risk with an attributable risk of 2.1 per 1000 which was not statistically significant.

These findings coincide with a Scandinavian study which did not find a link between CLL and pesticide exposure [*3*]. Our study may indirectly support the conclusions of an Italian study that suggested that the high risk of CLL and low-grade NHL in farming may be due to exposure to animal transmitted diseases [*9*].

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