Attachment #1

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A CLUSTER OF HODGKIN'S DISEASE IN A SMALL COMMUNITY: EVIDENCE FOR ENVIRONMENTAL FACTORS

Shorter Title: A CLUSTER OF HODGKIN'S DISEASE EVIDENCE FOR ENVIRONMENTAL FACTORS

Running Title: HODGKIN'S DISEASE

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ABSTRACT

A cluster of Hodgkin's Disease (HD) cases occurred in a small rural town of 1,250 people. Ten cases of HD and four cases of non-Hodgkin's lymphoma were identified within this town since 1954. This town therefore had an average annual incidence and mortality for HD of 41.3 and 22.5 cases per 100,000 population respectively. Most cases of HD demonstrated case-contact associations, and a distinct geographical distribution. The data suggested that there was an environmental agent responsible for the elevated rates of HD. One unique aspect of this cluster is that this town has only one industry, a large grain elevator. The cases closely surrounded this elevator. We postulate that residents of the town are subject to chronic immune stimulation from mitogenic substances in this environment. These agents may alter immunity in the residents of this community and predispose them to HD.

INTRODUCTION

Recent interest in the epidemiology of Hodgkin's disease (HD) is based on the studies by Vianna et al.[1] which detected an increased incidence of this disease among classmates and their contacts within a high school in Albany, New York. Subsequent reports have described other clusters of Hodgkin's disease,[2,3,4,5] with some evidence suggesting an increased incidence among schoolteachers[6] and physicians.[7,8] These studies have been criticized and/or invalidated on statistical grounds.[9-13] No clusters of Hodgkin's disease have been thus detected in carefully investigate random populations using time-space methods.[14,15] Furthermore, the previous studies involved large, unstable populations which would not allow examination of environmental factors. In 1974, we became aware of a small rural community in mid-Michigan reported to have a high incidence of Hodgkin's disease. This prompted our investigation which has produced suggestive evidence that environmental factors are etiologically important in HD.

METHODS

Our index case of Hodgkin's disease was identified as a patient on a general medical ward of the University Hospital. She related that a number of residents of her small town of 1,200 people had developed Hodgkin's disease or lymphoma over the last 20 years. This prompted the current epidemiologic

study. Other cases of Hodgkin's disease and lymphoma were then found by contact with local physicians, questioning of all individuals with lymphoproliferative diseases and their family members, and survey of local hospital records. In addition, the entire file of county death certificates over the last 40 years was surveyed (18,977 death certificates).

Once a case was identified, questionnaires were distributed to the individual and all living family members by a community volunteer unrelated to any of the cases. The questionnaire, which included information on ancestry, residence, education, hobbies, pets, employment, medical history, religion, blood transfusions, and family history was sufficiently broad to identify possible genetic and environmental factors and relationships between cases. Following completion of this stage of the study, the investigators interviewed all individuals known to have had Hodgkin's disease or other type of lymphoma or their closest living relative. The patients were asked to identify any and all relationships to the other patients and their respective relatives. In addition, all previous residences of each case were identified on a map of the community.

No completely satisfactory way has been developed to determine the statistical significance of established linkages among individu in studies such as ours.[1] However, to determine if interrelationshi and geographic patterns detected in the study could occur by chance alone, two control groups were questioned about interactions amongs themselves and previous residences within the town. Both groups we age, sex, and race matched to the patients. One control group was

chosen randomly from locations within the community; while the other control group consisted of individuals matched for location of residence near the patients with Hodgkin's disease.

Descriptive and sociologic data pertaining to the town were obtained from published materials made available by local and county sources as well as direct field survey. All population statistics were obtained from the United States Bureau of the Census Reports, 1950-1970.[16,17] The local Department of Water and Power provided detailed maps of the water supply with all modifications.

Average annual crude incidence and mortality rates of Hodgkin's disease and non-Hodgkin's lymphomas were calculated for the 20 year period, 1954-1973, using the average community population during this period in the formula (rate = # cases [1954-1973]/ 20 x average population corrected to a population of 100,000). Average crude mortality rates for a variety of malignancies which occurred in the county and town were similarly computed using data obtained from the county death certificates. Incidence and mortality rate ratios were computed using the 1970 U.S. caucasian population as the referent; these ratios were standardized for age using the age distribution of the town as a standard.[18] All calculations exclude the index case except where noted. The small number of cases precluded reliable interpretation of age specific incidence and mortality rates.

Hospital and medical records of all cases were reviewed and utilized to clinically stage the Hodgkin's and lymphoma patients according to accepted criteria.[19] Determination was also made as accurately as possible in a retrospective study of the date of onset of disease defined as onset of clinical or physical

symptomatology rather than date of diagnosis. Original pathological material was reviewed in eight of the ten cases of Hodgkin's disease and three of the four cases of lymphoma with positive confirmation. Pathological material was no longer available for two cases of Hodgkin's disease but had been reviewed at outside institutions at the time of diagnosis (case C - Michigan State Health Department; case E - Saginaw Osteopathic Hospital). Biopsy material was obtained for case A only from 1972. Similarly, biopsy material was not available for one case diagnosed as non-Hodgkin's lymphoma. In these three cases, clinical history and pathological reports, however, were indicative of appropriate diagnosis. Pathological specimens were classified according to the histopathological classification scheme proposed by Berard[20] for Hodgkin's and non-Hodgkin's lymphoma.

From all the living cases in the cluster, blood was obtained for HL-A tissue typing[21] and serologic studies including serum antibody titers to Epstein-Barr virus capsid antigen (VCA) and nucleic antigen (EBNA), rubeola, and cytomegalovirus as determined by the indirect fluorescent antibody technique.

RESULTS

A. Description of the Town and County

The community is located in central Michigan in a county of 39,000 which is 99% caucasian. The county is rural with large areas devoted to agricultural use. Major crops include

sugar beets, corn, soybeans, and wheat. Ninety-five percent of the world's supply of navy beans (Phaseolus vulgaris L.) are grown in this part of central Michigan.

The community is a distinct rural town surrounded by farms. The nearest population center of 10,000 is located 10 miles away. The town's population is 1,257 and has been very stable with little emigration or immigration. The composition of the populace is nearly identical to that of the county in all respects includin age distribution, sex ratio, and ethnic groups and is not significantly different from the overall United States caucasian population (1970 census). The area of the entire town is less than one square mile and is divided by a highway into northern and southern halves (See Figure 1). Thirty-six percent of the homes are located in the northern section, the remainder being in the southern half. However, forty-one percent (512) of the town's population live in the northern half (expected 36%). Water supplies to both sections are derived from a common source.

The town contains only one industry, a large bean and grain elevator which has been present at the same site within the northern sector since 1909 but has undergone major expansion progressively since 1957 and is currently the largest storage elevator in the state with approximately 3 million bushels capacity. For several decades, the commodities stored yearly have been 60% corn, 20% navy beans, 10% wheat, 9% soybeans, and 1% oats. The harvest begins in July and runs through October and during these months, the elevator emanates a cloud which covers the northern sector of the town with dust, particularly the surrounding two blocks.

The community high school, which serves the entire district, since 1920 has also been located on the north side of the town. Since 1967, however, the facility has been used for elementary grades only.

B. Incidence and Mortality of Hodgkin's Disease and Other Tumors

By the methods discussed, we were able to identify ten cases of Hodgkin's disease (including the index case) and four cases of non-Hodgkin's lymphoma developing in individuals havir contact with the town during the years 1954-1973. Contact was defined as including residency or daily work within the town prior to or at the time of development of Hodgkin's disease. We failed to document any cases of Hodgkin's disease which occurred within the town prior to 1954.

The United States ten cities survey of 1947[22] estimated Hodgkin's disease annual incidence rates of 3.5/100,000 for white males and 2.6/100,000 for white females. A more recent study[23] gave similar results for the years 1969-1971 with annu age-adjusted rates of 4.1/100,000 for white males and 2.7/100, for white females or 3.4/100,000 white population overall. Although rates for the state of Michigan are not available, th above study did survey incidence of Hodgkin's disease within the Detroit Standard Metropolitan Statistical Area (SMSA) whic included 47% of the state's population albeit emphasizing metr politan areas and detected similar rates for the caucasian population. The annual mortality rate for the disease is estimated to be 2.3/100,000 white males and 1.3/100,000 white females or 1.7/100,000 white population overall.[24]

Based on age and sex specific incidence rates, the number of cases of Hodgkin's disease expected to have occurred within the community studied during the 20 year period was 0.74. The age-adjusted average annual incidence rate within this population has been 41.3 cases per 100,000 which is 12.2 times the expected incidence rate. The average annual mortality rate was similarly increased at 22.5 deaths/100,000. Although incidence rates for the county are not available, review of the county death certificates yielded an annual average mortality rate of 2.6 per 100,000 for Hodgkin's disease (including the cases within the town). Mortality rates for a variety of other malignant states were similarly estimated for the town and county with all values being similar to or less than twice the expected rate[25] (Table I). County mortality rates for the period 1969-1974 determined from death certificate data correlated well with a published survey of mortality rates during the period 1950-1969.[24]

C. Onset and Mode of Presentation of the Cases

The date of onset and mode of presentation of the cases of Hodgkin's disease and lymphoma are presented in Table II. Only one case of Hodgkin's disease (case E) could not be specified as to approximate date of symptomatic onset. In making this tabulation, two dates are given for case A who was first diagnosed as having Hodgkin's disease in 1954 with stage I disease, was apparently cured with surgery and radiotherapy, and remained in remission until 1972 when, 10 months following his return to the town, he developed recurrent

Hodgkin's disease. Although recurrence of the disease after prolonged periods of time has been documented, such cases are very rare.[26] We therefore believe the diagnosis of Hodgkin's disease in this individual, 18 years after the initial diagnosis, represents a second primary tumor (in calculating incidence rates, this case has been counted only once).

The clinical presentation, histological subtype, genetic data, and virological data were similar in every respect to previous studies on Hodgkin's disease. Occupations of the cases showed no striking dissimilarity to other individuals in the town. A trend was seen in which eight of 10 cases had the onset of disease between November and February. This does not reach statistical significance.

D. Case Clustering

Figure 2 represents the case-contact interrelationships demonstrated in the study. The complexity of the diagram demonstrates the problem of recognition of important associations in a small town where "everyone knows everyone else". One of these relationships, however, is quite striking and merits further attention. Cases B and J are remarkable because they are the first known linkage via a blood transfusion. Case B donated one unit of blood which was transfused into case J's mother while she was three months pregnant with One year and four months after donating the blood, B developed Hodgkin's disease. Case J was well until age 16 when he also developed the disease.

Residence in the northern sector of the town near the grain elevator and high school correlated with the development of Hodgkin's disease or lymphoma. The residence location of each case is shown on a map of the town (Figure 1) with all residences of these individuals for the year 1950-1973 being shown. Multiple residences are indicated where appropriate. Eight cases (six Hodgkin's disease and two lymphoma) had resided in close proximity to the elevator either prior to or at the time of diagnosis of disease. This was in contrast to the southern sector of the town where most of the homes in the community are located but which was associated with development of only three cases (E, F, and N) of these diseases. Attention is focused on the grain elevator because of its size and location. The striking spatial relationship of Hodgkin's disease within this community may represent a "cluster" within the larger cluster reported in this paper.

The control groups demonstrated multiple interlinkages similar to the Hodgkin's patients. However, of 15 randomly chosen individuals residing in the southern sector of the town, only four could be linked to the area near the grain elevator during the years 1954-1973. This suggests that clustering of Hodgkin's disease cases in the northern sector of the community may not have occurred by chance, but this was not statistically significant.

DISCUSSION

The cause of Hodgkin's disease is not known. The geographic variation in frequency of the disease seen in international comparisons,[27] association with socioeconomic status,[27] and epidemiologic patterns based on urban-rural character of the region[28] suggest, however, the importance of environmental factors.[27-30] A seasonal trend in onset of clinical Hodgkin's disease with a peak in December and January also supports this concept.[13,31,32] Previous investigations of Hodgkin's disease clusters have unfortunately dealt with large migratory populations, thus precluding assessment of critical personal contacts and identification of environmental agents. The geographic and sociologic features of the cluster described in this report, however, offered an opportunity to investigate factors as might be of etiological importance.

Epidemiologic studies of Hodgkin's disease clusters have suggested the possibility of horizontal transmission as might be expected of an infectious disease.[1-7] We therefore placed great emphasis on determining interpersonal contacts and interrelationships in our study. Both the control and patient groups demonstrated similar interlinkages. The association, however, between cases B and J is of interest because this is the first reported case of a relationship of blood transfusion to the development of Hodgkin's disease. This suggests that vertical transmission may also be possible.

An unexpected finding of this study was the apparent correlation of residence in the northern sector of the town

which surrounds the grain elevator with Hodgkin's disease developme This distribution of cases suggests an environmental agent. Navy beans, which compose 20% of the elevator's storage capacity, may be of importance in giving this community its unique features. These beans are known to contain phytohemagglutinin (PHA), a mitogen whic selectively stimulates thymus-derived lymphocytes (T cells) to unde blast transformation and replication.[33] The residents of the north sector of the town are exposed to dust, which may represent a form chronic antigenic or mitogenic stimulation. Other grains are also stored in this elevator but in lesser quantities which probably contain other lectins.

We have recently measured the lymphocyte transformations of community residents and control populations to a slurry of navy beans as well as PHA. These results have been briefly reported elsewhere.[34] Preliminary results suggest that the residents surrounding the grain elevator have significantly enhanced stimulation to both agents as compared to similar non-resident control groups. This suggests stimulation of thymus-derived lymphocytes of the town's residents to lectins in the bean dust.

Antigenic stimulation as a factor of lymphoma development does not exclude the possibility of other agents such as oncogenic viruses play an etiologic role. It is possible that both antigenic stimulation and viruses must be present to result in neoplastic transformation.[35-37] Transformed lymphocytes are known to support the replication of viruses[38,39] and are required for the induction of leukemia by RNA tumor viruses.[40] Thus, chronic antigenic stimulation and resultant lymphocyte transformation might provide an environment favorable to oncogenic viral replication.

The effect of chronic exposure to mitogenic substances on humans is not known. It is known, however, that inclusion of raw navy beans in animal diets may lead to severe depression of growth and even death.[41] Andrews et al.[41] have demonstrated that the toxic agent in raw navy beans is indeed PHA. Dusts containing this agent may similarly prove to be toxic to humans in ways not well understood at the current time.

In addition to explaining the unusual distribution of Hodgkin's disease cases within the community, the hypothesis of chronic antigenic exposure may also explain the clustering of disease onset during the months of November through February following the summer and fall harvest and maximal exposure to bean dusts. The development of Hodgkin's disease in two cases, four and ten months following exposure to the town, provide further evidence to suggest an environmental agent. Our study indicates this agent may induce disease after an incubation period which varies from several months to 16 years. This conclusion is in agreement with previous estimates.[2,42]

We have postulated that chronic immune stimulation may predispose susceptible individuals to development of Hodgkin's disease. Although not proven, it is our hope that this hypothesis may provide new insight into the pathophysiology of the disease, suggest experimental approaches of study, and stimulate reassessment of previous clusters of HD and leukemia for evidence of similar environmental agents. Although "navy bean" dusts are probably unique to this town, it may be likely that many other environmental agents elsewhere also result in antigenic stimulation of the immune system.

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TABLE I.

AVERAGE ANNUAL MORTALITY RATES

(per 100,000 population)

Tumor	County (1969-1974)	Town* (1955-1974)	Expected (1969)[25]
Lung	20.4	41.0	30.6
Breast (female)	26.2	26.2	29.1
Cervix (female)	5.4	0	6.7
Colon	13.9	31.9	17.2
Prostate	14.3	19.4	16.6
Pancreas	4.2	9.1	8.7
Bladder	4.2	4.5	4.2
Hodgkin's disease	2.6*	\22.2	1.7
Non-Hodgkin's Lymphoma	3.9*	\ 4.5	2.0
Reticulum Cell Sarcoma	3.9*	\ 4.5	1.2

*1955-1974 - calculated from the average annual population (this has remained relatively stable throughout the study period).

TABLE II.

HODGKIN'S DISEASE

Case	Sex	Date Onset	Age at Onset	Mode of Presentation	Histological Type
A	M	6/54 1/72	21 39	R. cervical IV B	--- Nodular sclerosing
B	M	5/57	32	Bilateral cervical	Lymphocyte predominance
C	F	2/58	65	Cervical	Unknown
D	M	1/61	12	L. cervical	Nodular sclerosing
E	M	?	?	Generalized, IV	Unknown
F	M	12/67	15	R. cervical	Nodular sclerosing
H	F	12/71	52	Inguinal mass	Lymphocyte predominance
I	F	11/72	22	III B	Mixed cellularity
J	M	2/73	16	III B	Mixed cellularity
K	M	11/73	66	IV	Lymphocyte depletion

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