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CLINICAL EVENTS SUGGESTING HERPES-SIMPLEX INFECTION BEFORE ONSET OF BURKITT'S LYMPHOMA

A Case-control Study in West Nile, Uganda

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Summary Thirty cases of Burkitt's lymphoma and thirty age and sex matched controls were interviewed in West Nile, Uganda. Ten of the cases, but only one of the controls, had a history of oral vesicles or ulcers or an injury at the site of the tumour within the preceding 3 months ($P<0.01$). Three more cases gave a history of recent unilateral conjunctivitis, "measles", or a high fever just before tumour onset. These histories are compatible with *Herpes simplex* infection just before tumour onset, and the possibility of this or another virus acting as a trigger for the tumour is discussed. Serological and virus isolation studies are in progress using materials collected during the study.

Introduction

Burkitt's lymphoma, like some tumours of viral origin in animals, may occur simultaneously at multiple sites. In addition, clusters of cases associated temporally and geographically occur in some parts of the world such as West Nile, Uganda,[1-3] and help to strengthen the impression that the tumour is caused or triggered by an infectious agent. The two agents

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most frequently proposed have been Epstein-Barr (E.B.) virus and malaria; patients with Burkitt's lymphoma have high titres of antibody against E.B. virus, and the tumour is most common in parts of the world having holoendemic malaria.[4,5] In many parts of West Nile District, however, 70–80% of the children have positive malaria smears[6] and 90% over age 3 have measurable serum E.B. virus antibodies[7]: clearly, further information is required to explain the occurrence of the tumour in about 20 individuals per year of the 572,000 persons in the district.

In connection with broader epidemiological and serological studies in West Nile, we have had the opportunity to interview in depth the families of Burkitt's lymphoma cases soon after onset of the tumour. The findings suggest that the tumour is preceded, in a remarkable number of cases, by clinical signs of infection or by an injury at the same site as the tumour. Interviews of randomly selected matched controls indicate that these findings are not due merely to a high incidence of infections in the population.

Patients, Controls, and Methods

West Nile, a district of 4147 sq. miles (10,600 sq. km.) in the north-west corner of Uganda, is the home of the Lugbara, Alur, and Kakwa people. Almost all are subsistence farmers, and the largest town, Arua, has a population of 11,000. The population density (138 per sq. mile) has nearly doubled in the past 20 years.[8] West Nile has a moderate rainfall, 35–70 in. (88–176 cm.) per year, and the area with most cases of Burkitt's lymphoma is at an altitude of 2000–5000 ft. (600–1500 m.).

The district is served by three Government hospitals, several mission hospitals, and over thirty Government dispensaries. Almost all cases of Burkitt's lymphoma reaching medical attention become known to the W.H.O. Burkitt's Lymphoma Project in Arua. Most of them are seen at Kuluva Hospital, where treatment is offered by the project. During the period August, 1972, to July, 1973, eighteen West Nile patients with Burkitt's lymphoma were seen, and their families were interviewed within 3 months after the onset of the tumour. The families of twelve less recent, living cases, whose tumours had occurred since December, 1970, were similarly interviewed.

The diagnosis was established by biopsy and/or touch preparation in twenty-seven of the thirty cases, by finding tumour cells in the cerebrospinal fluid in one, by needle aspiration smear of the liver in one, and by the clinical picture and response to cyclophosphamide in one case. The biopsies were read by the Pathology Department of Makerere University, and the touch preparations and aspirate were read by A. G. D. and E. H. W.

Fifty of the sixty interviews (thirty cases and thirty controls) were conducted by A. G. D. with the help of a team of paramedical workers acting as interpreters, and the last ten control interviews were completed by this team alone. Three interpreters were used, one each for the Lugbara, Kakwa, and Alur languages. All of the patients were Lugbara, except two Kakwas and two Alurs.

Each interview began with a general question such as "How did the disease (tumour) begin?" Only after the information so elicited had been expanded and recorded were direct questions asked. For example: "Has the patient ever had malaria? Has he ever had measles? Has he had a mouth infection, or a sore or red mouth? Has he had blisters or sores on the lips or in the mouth? Has he had pneumonia, a chronic cough, an injury, and so on?" The interviews were conducted from a printed questionary on which the answers were recorded.

To establish the incidence of similar events in the general population, a control individual was interviewed for each case. West Nile is divided into subcounties. Those in which cases have occurred range in size from 80 to 450 sq. km. and in population from 9000 to 29,000.[8] Every adult male is enrolled on tax lists at the subcounty headquarters. The completeness of enrolment has been verified by the teams working in the W.H.O. project and approaches 100%. The location of the home of each case was known exactly, and for each one a control was selected from the tax list in the same subcounty, using consecutive numbers from a random-numbers table. Two cases came from a few miles over the Zaire border, and the adjacent Uganda subcounty was used in choosing their controls. The home of the man selected was visited, and a child of the same age and sex as the case was sought. If the family did not have a suitable child, the next household to the north was visited, and so on until a control of the right age and sex was obtained. Contingency directions, such as proceeding east rather than north if a parish boundary was reached, were provided in advance.

In two cases, refusal to cooperate made selection of a second random number necessary, and a few houses were bypassed because the inhabitants were absent, but otherwise the statistical rules were followed absolutely. Each control family was interviewed in the same manner as the case family, using particular care to elicit a history of stomatitis, recent injuries, and so on. Unfortunately, conjunctivitis was not included in the early questionaries, so that the history of eye infections is not completely controlled. After the team assisting with interviews had done fifty interviews, they were felt to be at least as thorough as A. G. D., and the last ten interviews of control cases were done without him.

The accuracy of the translations was assured by several factors. The Lugbara interpreter has spent 4 years in Burkitt's lymphoma work and is quite familiar with the

EVENTS WITHIN THE 3 MONTHS BEFORE ONSET OF BURKITT'S LYMPHOMA

BL series no. (case)	Sex	Age	Site of lymphoma	Antecedent events
185	M	11	L temple, parotid, and submandibular areas	Small vesicles " filled with water " on lips 2 wk. previously
195	F	4	L maxilla	Small ulcers on gums near L maxilla 3 wk. earlier
196	M	6/7	All 4 quadrants of jaw involving gums; abdominal mass	Gum ulcers 1–2 wk. previously
199	M	7/8	L orbit and nasal cavity	Gingivitis and eye injury (see text)
200	M	9	Around upper second molar, slight L proptosis	Gingival ulcer (see text)
202	F	5	R mandible, ascites, huge L ovary, L flank mass	Conjunctivitis, pain in R lower jaw, ? ulcer (see text)
183	F	6	R maxilla and mandible	" Blisters with water inside " before age 4 yr.; hit in R parietal region 2 wk. previously
184	M	5	L mandible	Vesicular rash on lips and mouth 3 months previously (see text)
198*	F	8	R maxilla, R proptosis, massive enlargement of both ovaries	Red and itchy R eye 2 mo. earlier
186	M	45	L orbit	Abrasion to L eye 3 days earlier (see text)
201	M	9	L mandible, enlargement of L thigh	Kicked by cow at angle of L mandible 4 days previously; mouth bleeding suggested break in oral mucosa
174*	M	4/5	R mandible	High fever 2 wk. earlier; febrile illness in neighbourhood children
168*	M	10	R maxilla	Cough and chest pain 3 wk. earlier
191*	F	7	R orbit, L mandible, R ovary	Sore throat and cough a week or two previously
170*	M	10/11	Thyroid	History of bilateral conjunctivitis twice yearly
190*	F	6	Angle of R mandible, R ovarian mass	" Measles " with vomiting, diarrhœa, high fever, and red mouth 6 wk. earlier
187*	M	10	Mediastinal and hepatic	None, but sibling had gingivitis with vesicles near upper incisors and maculopapular rash on trunk, and neighbour's child had red stomatitis and high fever 2 mo. later
Control	M	10	..	" Small lumps, like scabies " at corners of mouth 3 wk. before interview

* Events in these patients were not systematically sought in the control group, so they were not included in the statistical comparisons.

disease and with medical terminology. A. G. D., although not a fluent Lugbara speaker, learned enough to check the accuracy of the questions. The occasional presence of other Lugbara interpreters during interviews offered further opportunities to verify the translation. Finally, since the same interpreters also worked with the control families, the differences between cases and controls are not due to translation difficulties.

Results

The age of the patients ranged from 3 to 45 years, all but two being children. Excluding these two adults, the average age was 6·4 years. There were twenty males and ten females. Eighteen were interviewed during primary treatment of the tumour, and for these the time between tumour onset and interview ranged from 12 days to 2·5 months. The other twelve were interviewed from 5 to 24 months after tumour onset.

Typically, the onset of the disease was described as a painless swelling of the jaw, orbit, or abdomen, often treated in vain with penicillin at a local dispensary before the patient came or was referred to the Burkitt's lymphoma project.

A number of Burkitt's lymphoma patients gave histories of recent medical events which might be of interest from the oncogenic point of view (see table).

Eight patients (nos. 185, 195, 196, 199, 200, 202, 183, and 184) had jaw or facial tumours which had been preceded by infections of the mouth, for example:

BL 199.—About Feb. 20, 1973, this boy had had gingivitis with blisters on the gums over the upper incisors. One week later he went to bathe in a nearby stream and returned saying something had hit him beside the left eye. The eye became red and painful. A few days later there was a slight swelling of the left face and proptosis of the left eye. This was at first treated with antibiotics, but by March 19 a polypoid mass in the left nostril was seen. On March 26 a biopsy showed Burkitt's lymphoma.

BL 200.—In March, 1973, according to the family, there was a small ulcer on the buccal gingiva opposite the last molar. This regressed, but reappeared about April 18. The grandmother with whom he sleeps noted a funny smell to the saliva and examined his mouth. There was a small ulcer in this location "with white fibres in it". A few days after this the area began to swell. A biopsy on May 5 showed Burkitt's lymphoma; the gingival mucosa at this time was intact.

BL 202.—In mid-March, 1973, all four children in this girl's family had had conjunctivitis affecting first one eye and then the other. The patient complained of pain in her right lower jaw a few days later, but the mother did not look in her mouth. About mid-March the mother noted a mass around the right lower canine and premolar teeth—the site of the previous pain—and the abdomen began to swell.

BL 184.—This boy had lived in another district (Bunyoro) up to February, 1972, when his family moved to West Nile. The mother had had vesicles on the lips during each pregnancy, and these occurred at about 3 months' gestation during the pregnancy in question. Occasionally, when ill, the patient also had had vesicles on the lips. In March, 1972, his mother and all five children, including the patient, had a vesicular rash on the lips and mouth. The swelling began 3 months later, in June, 1972.

Three patients (198, 199, and 186) had had unilateral conjunctivitis followed by an orbital and/or maxillary tumour, for example:

BL 186.—A child in this man's household had had vesicles on the lips after malaria 1 year previously. A neighbour's child had had similar lesions in July, 1972. In August, 1972, the patient, while drawing water, leaned over and accidentally abraded his left eye with a piece of grass. The eye became red and painful. The swelling began 3 days later.

Four patients (183, 186, 199, and 201) had had an injury to the site of the tumour just before tumour onset.

Six patients (see table) had an antecedent history of miscellaneous infections or contact.

The remaining thirteen patients cited no events of apparent significance in the months preceding tumour onset.

Among the thirty matched controls, only one similar history was obtained for the 3 months before interview.

In all, ten patients (compared with one control) had a recent history of oral lesions and/or an injury to the site of the tumour. Since *Herpes simplex*, type 1, frequently causes oral lesions, unilateral conjunctivitis, or a non-specific febrile illness, one might include the additional three patients with unilateral conjunctivitis, "measles", and a "high fever" just before tumour onset as part of those with a "significant" medical history, bringing the total to thirteen of the thirty cases. One patient (no. 199) had all three events—oral vesicles, an injury, and conjunctivitis—in the recent past.

In addition to the single control with "small lumps" at the corners of the mouth (possibly impetigo) within the 3 months before the interview, another had also had lesions at the corners of the mouth 6 months to a year previously, and three others had had oral lesions during measles 3–7 years before interview.

Several controls had had injuries, but none near the site of the tumour in the corresponding case.

Of the sixteen controls who were asked about conjunctivitis, three had had conjunctivitis in the previous 3 months, and ten had had the disease sometime during their lifetime. One of the three with recent conjunctivitis had had recurrent attacks of redness and tearing in one eye "whenever he had malaria". It seems that conjunctivitis is a common occurrence in West Nile children but that oral vesicles or ulcers are relatively uncommon.

Discussion

Before trying to explain the findings, one must be certain that they are real. We took great care to avoid influencing the histories by asking leading questions, and we do not think that this could account for the findings. It would take considerable imagination to suggest, consciously or unconsciously, that one patient was kicked in the jaw by a cow and that another patient's halitosis attracted his grandmother's attention to a gingival ulcer at the site of the tumour. There seemed to be no tendency to reply "yes" to every question merely to please the doctor, as is the habit in some cultures. Numerous emphatic "no's" were encountered in every interview, and reversal of a previous answer on further questioning was rare.

A more subtle source of error might be that after a child develops a tumour the parents tend to remember minor events around the site of the tumour with unusual acuity. This is true to some extent, but as far as can be estimated it did not play a major role. The people of West Nile spend a great deal of time close to their children, and the control families also seemed to remember minor illnesses in surprising detail.

Statistical evaluation of the findings is of limited benefit. Taking the gross figures of 10 cases of 30 with oral lesions or injuries versus 1 of 30 controls, the P value is less than 0·01. This, however, does not use the fact that most of the lesions or injuries described occurred at the exact site of the tumour

The odds against this occurring in a matched series of controls are orders of magnitude higher—indeed, none of the controls had a lesion or injury at the site of his matched partner's tumour. It does seem, then, that one cannot explain away the findings on the basis of chance.

H. simplex infection is a possible explanation for the oral and ocular symptoms. The oral lesions described by the parents are quite consistent with herpetic vesicles and ulcers, and unilateral conjunctivitis is a fairly common manifestation of *H. simplex* infection. Until serological and virus-isolation studies are complete, the cause of the herpes-like symptoms related by the patients remains speculative. Although *H. simplex* is the agent most urgently in need of investigation, it is possible that the oral lesions and/or conjunctivitis are manifestations of another virus or even of E.B. virus infection.

Approximately 200 sera and throat swabs for virological studies have been collected from nineteen cases and nineteen controls and their families, and laboratory work is now under way to detect the presence of *H. simplex,* E.B. virus, and other agents.

Seventeen patients had no history of injuries or symptoms possibly consistent with infection by *H. simplex,* possibly because of the minor nature of such symptoms in relation to the tumour itself. Not all parents bothered to investigate minor complaints such as pain in the mouth, and preceding events may have been forgotten in the months or years after tumour onset. Indeed "herpetic" histories or injuries were more common in patients interviewed within 3 months of tumour onset as compared with those interviewed later. Finally, since *H. simplex* infection is often inapparent, especially in young children, the absence of a positive history in some cases would not be surprising. What is surprising is that so many of the cases did give histories suggestive of *H. simplex* infection.

The role of injury may be to provide an entry point for an infectious agent (e.g., in cases 186 and 199), and mechanical trauma is a factor precipitating exacerbations of *H. simplex* lesions in chronically infected individuals.[9]

If *H. simplex* or another virus was responsible for the events preceding clinical onset of the tumour, were the oral ulcers and so on the first symptom of Burkitt's lymphoma or did they precede and perhaps trigger tumour development? In other malignancies,

especially those under treatment, *H. simplex* exacerbations are common,[10] and the same thing might be true with Burkitt's lymphoma. If this is the case, the effect would have to be a local one, because most lesions were at the exact site of the tumour, and this would not explain why the herpetic lesion heals before the tumour enlarges.

The short time between the lesions or injuries and appearance of the tumour might seem to rule out any casual role in development of the tumour, but Burkitt's lymphoma grows very rapidly, the cells having a doubling-time of about 25 hours.[11] In fine structure Burkitt's lymphoma cells resemble primitive or stimulated lymphoid cells (lymphoblasts). The tumour could be an extension of a normal process —immune response to an antigen—which has failed to be "turned off" by the mechanisms of control. In this case the triggering event (e.g., virus infection) might be one which interferes with "turning off" after lymphocytes have begun to proliferate at the site of another antigenic stimulus, thus explaining the short lag time before the tumour becomes visible.

Whatever the mechanisms, the clinical findings seem real enough—in West Nile, Uganda, Burkitt's lymphoma cases, more frequently than matched controls, give a recent history of oral vesicles or ulcers or a physical injury, and these events occur at the site of the tumour.

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