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Advance Access publication 19 January 2005

International Journal of Epidemiology 2005;**34**:132–137
doi:10.1093/ije/dyh339

Chronic hepatitis B virus infection and mortality from non-liver causes: results from the Haimen City cohort study

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Accepted 25 August 2004

Background Studies of chronic hepatitis B virus (HBV) infection in endemic populations have rarely documented causes of mortality other than liver disease and hepatocellular carcinoma (HCC).

Methods We analysed all-cause mortality related to HBV infection, focusing on the deaths not related to liver disease in a prospective cohort of adults living in Haimen City, China, who were followed from 1992 to 2002. Death certificate data from 4590 deaths among 83 794 individuals were analysed. At cohort entry, 15.0% of the 58 454 male subjects and 10.7% of 25 340 female subjects were hepatitis B surface antigen positive [HbsAg(+)]. HCC and chronic liver disease were the major causes of death in both men and women in this population. The analysis focused on non-liver causes of death.

Results When liver-related causes of death were excluded, there was still a significantly higher age-adjusted death rate among HBsAg(+) individuals. The relative risks (RRs) and 95% confidence intervals (CIs) for all non-liver deaths among HBsAg(+) subjects were 1.2 (1.1–1.3) in men and 1.4 (1.1–1.7) in women. Non-liver causes were further subdivided into cancer and non-cancer groups. For all non-liver cancers, the RR was 1.2 (1.0–1.4) for males and 1.7 (1.2–2.3) for females. Non-liver, non-cancer deaths had RRs of 1.2 (1.1–1.4) and 1.2 (0.9–1.6) in males and females, respectively.

Conclusions HBV-infected individuals may be at increased mortality risk from non-liver causes. Possible reasons include a direct effect of HBV infection, changes in the host immune system as a cause or effect of chronic infection, and behavioural factors associated with HBV infection. Further studies are needed to confirm this finding.

Keywords Hepatitis B virus, cohort study, mortality, China

The World Health Organization estimates that there are 400 million people chronically infected with hepatitis B virus (HBV) in the world today.[1] The long-term sequelae of chronic infection have been well documented in terms of its effects on the liver. Chronic carriers of HBV are at greatly increased risk of chronic active hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). Rarer conditions such as polyarteritis nodosa and membranous glomerulonephritis have also been associated with HBV infection.[2,3] The effect, if any, of chronic HBV infection on non-liver morbidity and mortality has, however, not been extensively studied. One previous study of hepatitis B surface antigen (HBsAg) positive blood donors in England and Wales found an excess in mortality from non-Hodgkin's lymphoma in males and deficits in deaths from circulatory diseases in both males and females compared with non-HBV-infected individuals.[4]

In this study we examined patterns of all-cause mortality, comparing HBsAg(+) and HBsAg(−) adults in an HBV endemic area, Haimen City, China. Serum HBsAg is a marker of current

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HBV infection. Because most HBV infection in endemic areas is acquired in infancy or early childhood, it is assumed that the infected individuals in this study had been chronically infected for at least two decades.

Materials and methods

The analysis used mortality data from a prospective cohort in Haimen City, China. Previous publications have described the assembly of this cohort and earlier analyses of HCC mortality.[5] The protocol was reviewed and approved by the Institutional Review Board of Fox Chase Cancer Center (FCCC), the Medical Ethics Review Group of Haimen City, and the Ethics Review Committee of the School of Public Health of Fudan University.

Study population

Haimen City, in Jiangsu Province, is located in the delta region of the Yangtze river, immediately adjacent to Qidong City and within the area of highest HCC mortality in China. Between February 1992 and December 1993, study teams from the Haimen City Anti-Epidemic Station travelled to villages in each of the 35 townships of Haimen City to enrol subjects in the prospective cohort study. At entry, each subject completed a one-page questionnaire and donated a 9.0 ml sample of blood by venipuncture. A total of 90 836 adult residents of Haimen City underwent enrolment screening. All participants gave informed consent to enrolment in the cohort study. The study was explained to groups of assembled villagers at each location before screening began, and residents who did not wish to participate were free to leave without being questioned about their reasons.

Questionnaire data collected included name, residence, date of birth, occupation, cigarette use, alcohol consumption (≥4 drinks/week), tea drinking (≥4 times per week), past pesticide exposure, drinking water source by decade from the 1960s to the 1990s, staple food by decade from the 1960s to the 1990s, history of acute hepatitis, history of jaundice, history of cirrhosis, and family history of HCC. For this analysis, occupation is coded as a binary variable, peasant vs non-peasant, and can be considered a surrogate for socioeconomic status. Peasants in general have fewer economic resources than other classes of worker (factory workers, functionaries, business people).

For the purposes of this analysis, 58 454 male subjects and 25 340 female subjects were included, to give a total of 83 794. Among the 7042 (7.8%) subjects excluded, 633 (9.0%) were outside the required age range of 25–64 years at study entry and 6409 (91.0%) were from townships of <1000 study population where mortality reporting was considered inadequate. Subjects who were lost to follow-up during the course of the study owing to dissolution of their work units or permanent moves to locations outside Haimen City no longer contributed data to the study after the time of their exit from Haimen City. In total, 5673 subjects (6.8%) were lost to follow-up before December 31, 2002.

Designation of causes of death

Subjects were followed from cohort entry until December 31, 2002 for vital status. Each of the 35 township hospitals as well as city-wide referral hospitals is required to report all deaths from any cause monthly to the Haimen City Center for Disease Control (HCCDC). These reports include information from health centres in each village within the township. On a yearly basis, reporting groups are evaluated by the Haimen City public health authorities for the completeness and accuracy of reporting. Death certificates list only one cause of death, considered the major cause.

The majority of death certificates are completed by doctors in hospitals: 68.8% from city-level referral hospitals and 26.1% from township-level local hospitals. Village doctors report 2.8% of deaths, and 2.3% are reported by others, usually family members. During the period of this study, age-standardized all-cause mortality rates have been decreasing in Haimen City as well as in the cohort group, reflecting improvements in health and socioeconomic conditions in this area of China.[6]

Twice per year, study personnel identify cohort members from village lists and verify death certificate information by contacting village and township doctors. For HCC cases, surviving family members were also contacted to provide missing information. Deaths of individuals who leave Haimen City for work purposes without being granted a permanent change of residency are still reported to local authorities, although reporting is often delayed. Cohort members who were granted permanent changes of residency were considered lost to follow-up as of the date of the residency change. Age-standardized all-cause mortality rates in the cohort were 25–30% lower than those reported for Haimen City overall, possibly reflecting a 'healthy enrollee effect' in the cohort assembly. Age-standardized mortality rates for females for the age groups included in the cohort are consistently lower than those for males, as they are in the Haimen City population as a whole. Life expectancy is higher for females in China, and mortality rates for female adults are consistently lower.[7]

Laboratory methods

Serum was separated from whole blood within 24 h of collection in the laboratories of the HCCDC. Samples were tested immediately for HBsAg using commercial enzyme immunoassay (Shanghai Kehua Biotechnology Ltd), serum alanine aminotransferase using the standard kinetic method, and serum alphafetoprotein using commercial enzyme immunoassay (Abbott Quantum, Abbott Laboratories, North Chicago, IL, USA). Research subjects were informed of the results of these tests.

Statistical methods

Statistical analysis was conducted using SAS version 8 (SAS Institute, Cary, NC, USA). Relative risks (RRs) of mortality by cause associated with HBsAg positivity were calculated using Cox proportional hazards models for males and females separately and including a term for age as a continuous variable in the model. Any risk factor other than HBsAg status was considered a potential confounder if inclusion as a covariate resulted in a change of >10% in at least one other parameter estimate in the model compared with when it was absent. Adjustment for discrepancies between FCCC and HCCDC HBsAg test results was achieved using induced relative risk models.[5,8,9] The induced relative risk model is used to account for measurement error by estimating the true distribution of a covariate based on a validation subsample. In this case the FCCC HBsAg test (Auszyme Monocolonal, Abbott Laboratories) served as the gold standard for the validation of a random

subset of results. Compared with this standard, the HCCDC HBsAg test had 93.1% sensitivity and 94.3% specificity. These parameters were used to reallocate person-years of observation according to the expected value of the HBsAg test, a process that resulted in more conservative estimates of the RRs because it tends to make the groups more like each other. The estimated RRs for all-cause mortality without the use of the adjustment were 21% higher in males and 30% higher in females.

Results

In total, 4590 deaths occurred between cohort entry and December 31, 2002. In this period, 782 599 person-years of follow-up were accumulated. Table 1 shows the numbers of deaths and crude mortality rates by major cause. HCC and chronic liver disease are the leading causes of death in this cohort, as they are in the adult population of Haimen City in general, accounting for more than 20% of reported deaths.

Table 2 shows the RRs for HBsAg(+) vs HBsAg(−) males and females by cause of death. Age-adjusted death rates were 3.6 times higher (95% CI 3.4–3.9) in HBsAg(+) males and 3.0 times higher (95% CI 2.5–3.5) in HBsAg(+) females. Most of this excess mortality was due to HCC and chronic liver diseases. Combining all liver-related causes, HBsAg(+) individuals were at 16–22 times higher risk than HBsAg(−) individuals. Even after liver-related causes were excluded, HBV-infected males still had an RR of 1.2 (95% CI 1.1–1.3) and HBV-infected females an RR of 1.4 (95% CI 1.1–1.7).

Among males, there were significant excesses of both cancer (RR 1.2, 95% CI 1.0–1.4) and non-cancer (RR 1.2, 95% CI 1.1–1.4) deaths of similar magnitude once liver-related causes were excluded. Examination of individual causes showed a significant excess only for stroke deaths (RR 1.4, 95% CI 1.1–1.8). Although the RRs for other causes were mostly >1.0, the 95% confidence intervals included 1.0. Only traffic accident deaths were associated with a lower risk in the HBsAg(+) vs HBsAg(−), but this was not statistically significant (RR 0.8, 95% CI 0.5–1.3).

For females, there was a significant excess of non-liver cancer deaths (RR 1.7, 95% CI 1.2–2.3), but the increase in non-cancer, non-liver deaths was not significant (RR 1.2, 95% CI 0.9–1.6). Examination of individual causes of mortality revealed no significant differences between HBV-infected and uninfected individuals, probably reflecting the smaller numbers of deaths overall in the female cohort.

Adjustment for risk factors other than age, also shown in Table 2, did not substantially change the RR estimates for the HBsAg(+) subjects compared with the HBsAg(−) subjects. As expected, lifestyle factors such as smoking, alcohol consumption, and occupation were significant risk factors for many of the causes of death, but because the distribution of these did not differ substantially between the infected and uninfected individuals, they did not confound the unadjusted RRs for HBsAg status.

Figure 1 shows the age-specific mortality rates for non-liver-related causes by gender and HBV status. Although the numbers of deaths made it difficult to definitively test the hypothesis formally, it appears that the excess non-liver mortality in HBV-infected individuals did not change greatly with age on a relative scale. The absolute mortality rates, of course, increased with age in all groups. For non-liver cancers, however, the excess mortality in HBV-infected individuals was confined largely to the younger age groups. Figure 2 shows the age-specific non-liver cancer mortality rates by gender and HBV status. HBsAg(+) males have excess non-liver cancer mortality only in the age groups <55 years old. HBsAg(+) females have excess non-liver cancer mortality up to age 65 years.

Discussion

The effect of chronic HBV infection on risk of HCC and chronic liver disease has been documented in many populations, but

Table 1 Number of deaths and crude mortality rate by cause, male and female HBsAg(+) and HBsAg(−) separately

	Male HBsAg(+)		Male HBsAg(−)		Female HBsAg(+)		Female HBsAg(−)	
	No.of deaths	Crude rate per 100 000 per year	No of deaths	Crude rate per 100 000 per year	No. of deaths	Crude rate per 100 000 per year	No. of deaths	Crude rate per 100 000 per year
Person-years	77 649		471 561		24 463		208 926	
All deaths	1468	1890.6	2480	525.9	189	772.6	453	216.8
Liver-related deaths	1111	1430.8	421	89.3	118	482.4	45	21.5
Hepatocellular carcinoma	812	1045.7	316	67.0	78	318.9	32	15.3
Chronic liver disease	299	385.1	105	22.3	40	163.5	13	6.2
Non-liver deaths	357	459.8	2059	436.6	71	290.2	408	195.3
Non-liver cancers	135	173.9	849	180.0	36	147.2	163	78.0
Stomach cancer	38	48.9	223	47.3	5	20.4	35	16.8
Lung cancer	47	60.5	304	64.5	5	20.4	39	18.7
Oesophageal cancer	11	14.2	104	22.1	3	12.3	13	6.2
Non-liver, non-cancer deaths	222	285.9	1210	256.6	35	143.1	245	117.3
Stroke	60	77.3	284	60.2	10	40.9	72	34.5
Chronic respiratory diseases	36	46.4	337	71.5	6	24.5	41	19.6
Cardiovascular diseases	16	20.6	87	18.5	4	16.4	20	9.6
Tuberculosis	10	12.9	37	7.9	1	4.1	3	1.4
Diabetes	6	7.7	21	4.5	2	8.2	7	3.4
Traffic accidents	14	18.0	128	27.1	3	12.3	35	16.8

Table 2 Relative risks for HBsAg(+) vs HBsAg(−) individuals, by cause of death

	Males		Females	
	Age-adjusted[a]	Adjusted for other risk factors[b]	Age-adjusted[a]	Adjusted for other risk factors[b]
All deaths	3.6 (3.4–3.9)	3.4 (3.1–3.6)	3.0 (2.5–3.5)	2.9 (2.5–3.4)
Liver-related deaths	18.9 (16.6–21.4)	15.9 (14.0–18.1)	20.9 (14.3–30.5)	18.9 (12.9–27.7)
Hepatocellular carcinoma	23.2 (19.8–27.2)	20.0 (17.1–23.5)	25.8 (15.8–42.2)	23.9 (14.6–39.2)
Chronic liver disease	11.7 (9.4–14.6)	9.3 (7.5–11.6)	14.4 (7.9–26.4)	13.1 (7.2–24.1)
Non-liver deaths	1.2 (1.1–1.3)	1.2 (1.1–1.3)	1.4 (1.1–1.7)	1.4 (1.1–1.8)
Non-liver cancers	1.2 (1.0–1.4)	1.2 (1.0–1.4)	1.7 (1.2–2.3)	1.7 (1.2–2.3)
Stomach cancer	1.3 (0.9–1.7)	1.3 (0.9–1.7)	1.0 (0.4–2.4)	1.0 (0.4–2.4)
Lung cancer	1.2 (0.9–1.6)	1.2 (0.9–1.6)	1.4 (0.7–2.9)	1.4 (0.7–2.9)
Oesophageal cancer	0.7 (0.4–1.3)	0.7 (0.4–1.3)	1.7 (0.7–5.6)	1.7 (0.7–5.6)
Non-liver, non-cancer deaths	1.2 (1.1–1.4)	1.2 (1.1–1.4)	1.2 (0.9–1.6)	1.2 (0.9–1.7)
Stroke	1.4 (1.1–1.8)	1.4 (1.1–1.8)	1.3 (0.7–2.2)	1.3 (0.7–2.2)
Chronic respiratory diseases	1.0 (0.7–1.3)	0.9 (0.7–1.2)	1.3 (0.6–2.6)	1.3 (0.6–2.6)
Cardiovascular diseases	1.2 (0.8–2.0)	1.2 (0.8–2.0)	1.6 (0.6–4.2)	1.6 (0.6–4.2)
Tuberculosis	1.4 (0.7–2.8)	1.4 (0.7–2.7)	1.8 (0.2–17.8)	1.8 (0.2–17.8)
Diabetes	1.7 (0.7–4.0)	1.7 (0.7–4.0)	2.0 (0.5–9.1)	1.6 (0.3–7.3)
Traffic accidents	0.8 (0.5–1.3)	0.8 (0.5–1.3)	0.9 (0.3–2.1)	0.9 (0.3–2.1)

[a] Adjusted for age only.

[b] Adjusted for age, smoking, occupation, acute hepatitis history, family history of hepatocellular carcinoma, drinking water, and staple food.

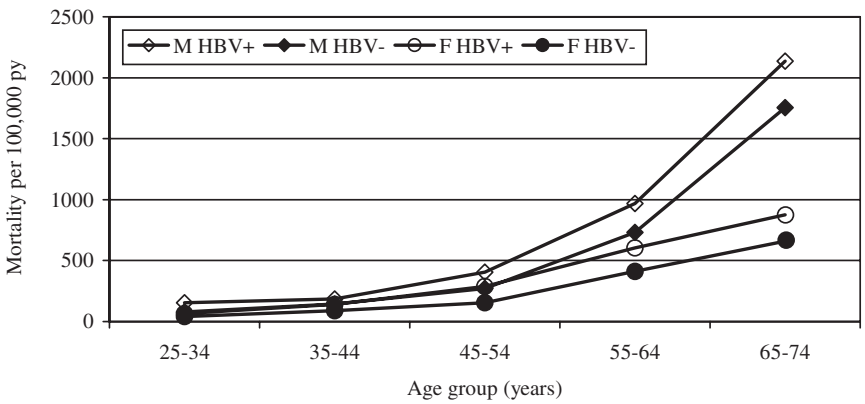

Figure 1 Age-, gender-, HBV-status-specific mortality rates for non-liver-related causes of death in the Haimen City cohort

excess mortality from non-liver causes has rarely been studied. There are an estimated 400 million individuals chronically infected with HBV in the world today,[1] and 600 000 HBV-related deaths occur annually worldwide.[10] We began this study with the aim of estimating the impact of HBV infection on non-liver causes of mortality, which could arise as a direct result of viral infection, as a result of indirect effects of chronic infection in reducing resistance to other diseases, or as an artefact of misclassified causes of death among HBV-infected individuals. Crook *et al*.[4] have published an analysis of mortality in HBsAg(+) blood donors in England and Wales in which they report, in addition to HCC and liver disease mortality, excesses of non-Hodgkin's lymphoma deaths in males (attributed to HIV infection) and deficits of cardiovascular and circulatory system deaths in both men and women. We expected that the patterns of mortality might be quite different in our study of a highly endemic population in China where HCC is the leading cause of death in adults. Indeed, HCC and liver disease deaths accounted for a much larger proportion of deaths in the Haimen City cohort than in the HBsAg(+) blood donors studied by Crook *et al*.

We found consistent excesses of non-liver-related mortality in both males and females chronically infected with HBV in Haimen City. There are many possible explanations for this observation, not the least of which is the possibility of misclassification of causes of death. Studies based on death certificates alone are likely to be biased towards the null because of the underreporting or misreporting. Cohort members were all informed of their HBV infection status at the time of cohort entry, but there is no known reason why non-liver causes of death would be more likely to be reported for decedents known to be HBsAg(+). Despite the potential for non-differential misclassification of causes of death, we believe that this report is important both for its public health implications and for the clues it may give as to the potential non-liver effects of chronic HBV infection. From the public health perspective, the data

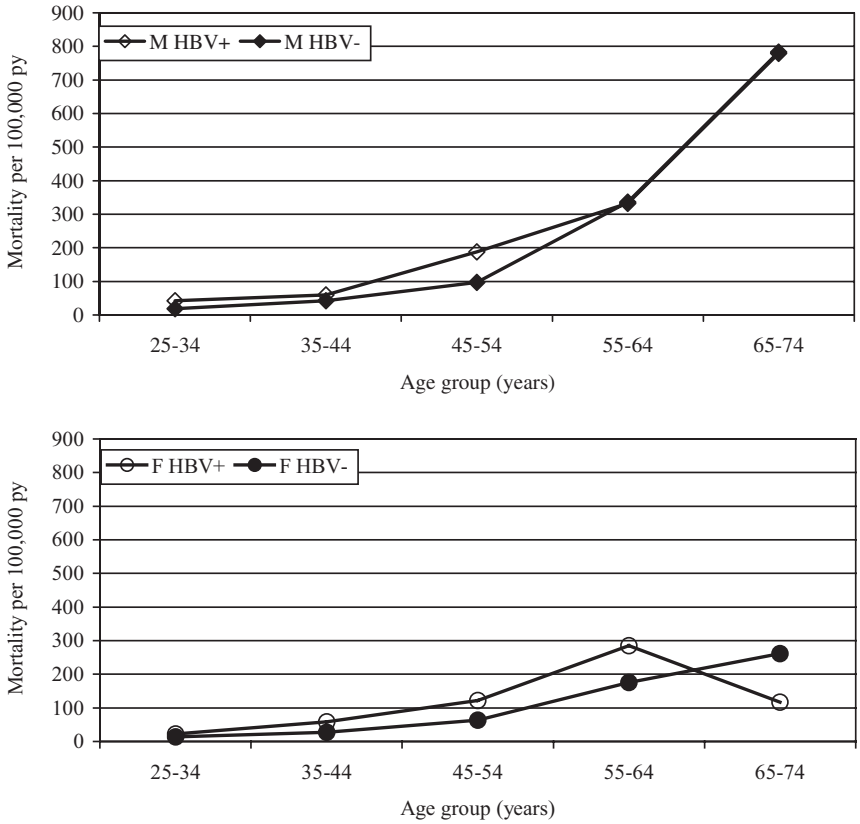

Figure 2 Age- and HBV-status-specific mortality rates for non-liver cancers for males (top panel) and females (bottom panel) in the Haimen City cohort

from the Haimen City cohort will aid in the estimation of the overall impact of HBV infection on mortality in endemic populations. This is an important issue in the planning and implementation of vaccination and in cancer and liver disease prevention, as well as in treatment programmes in areas where health-care funds are scarce. Our conclusions are strengthened by the size of the cohort, the extended duration of the follow-up, and the cohort's stability over time and low rate of loss to follow-up. Our study also includes substantial numbers of women chronically infected with HBV, which is rare in cohort studies of HBV epidemiology.

Our analysis is, however, based on death certificates where only one major cause and no underlying causes of death are listed. It is not known whether some of the individuals who died of non-liver causes had existing liver disease or HCC at the time of death that may have contributed to their demise. The excess of stroke deaths in men, for example, suggests a possibility that coagulopathy due to advanced cirrhosis may have increased the rate of haemorrhagic strokes in HBV-infected individuals. Additional field studies would be required to test this hypothesis. It is also possible that some of the other major cancers that occurred in this cohort may have either been misdiagnosed or represented metastatic HCCs. Although most HCC cases are not treated surgically in Haimen City, stomach and oesophageal cancer patients are quite often considered candidates for surgery, so it seems unlikely that these would be misdiagnosed frequently.

The increased mortality of HBsAg(+) subjects from non-liver causes may also reflect shared susceptibility factors. The establishment of chronic HBV infection requires the evasion of the host immune system through the early induction of immune tolerance or a deficiency in the host immune cells' ability to recognize and/or activate in response to viral antigens.[11] The immunological deficits that occur as either a cause or an effect of chronic HBV infection may have implications for diseases of other organ systems. HBV immune complexes are implicated in the aetiology of some cases of polyarteritis nodosa and membranous glomerulonephritis.[2,3,12] Other, more subtle, immune deficiencies may explain increased susceptibility of HBV-infected individuals to other diseases. It is also possible that lifestyle factors—for example, poorer nutrition and increased susceptibility to the effects of environmental toxins—play a role in increasing the susceptibility of HBV-infected individuals to many diseases.

In this study of HBV-infected individuals who are naïve to antiviral agents or interferons, we have shown that the population-level impact of chronic HBV infection appears to extend beyond those diseases usually considered to be caused by this infection. The subtle but broad effects observed in our study population suggest that HBV-infected individuals may have increased susceptibility to other disease processes. It also confirms, however, that HCC and liver disease are still the major health concern for HBV carriers, accounting for 76% of reported deaths in males and 63% of reported deaths in females. In planning efforts to reduce the morbidity and mortality from

HBV-related causes in endemic populations, a continued focus on hepatic disease is appropriate, but the impact on other causes of death should not be ignored. Further investigations are required to discover the causes of the excess of non-hepatic mortality observed in this population. It was found during follow-up that antiviral therapies for HBV had been administered extremely rarely in this cohort, and no antivirals were available in the area at the time of cohort assembly. It is not known what impact their use might have on non-liver-related mortality in other populations where HBV is endemic.

Acknowledgements

This work was supported by US Public Health Service grants CA-40737, CA-90395, and CA-06927 from the National Institutes of Health, a grant from Bristol-Myers Squibb Pharmaceutical Research Institute, and an appropriation from the Commonwealth of Pennsylvania.

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