Research Article

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Aspirin for Preventing Hemodialysis-associated Chronic Hepatitis C Infections

Hemodiyaliz İlişkili Kronik Hepatit C Enfeksiyonlarına Karşı Korunmada Aspirin

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ABSTRACT

Objectives: This study aimed to evaluate whether aspirin therapy is effective in protecting against hepatitis C virus (HCV) infection in maintenance hemodialysis patients, one of the high-risk groups for HCV infection.

Materials and Methods: This retrospective cross-sectional study included 408 patients with end-stage renal failure who underwent maintenance hemodialysis for at least 3 months in four private hemodialysis units in Hatay, Turkey, in January 2017. The patients were classified into two groups according to their aspirin exposure status: non-users (n=228) and regular aspirin users (n=180). The proportion of patients with hemodialysis-related chronic hepatitis C (CHC) was compared between the groups. Irregular aspirin users, patients infected with HBV or diagnosed with CHC before initiation of hemodialysis therapy were excluded from the study.

Results: The prevalence of hemodialysis-related CHC was 3.9% among the 408 patients. Hemodialysis-related CHC was not seen in any of the 180 regular aspirin users. Regular aspirin users showed a significantly lower prevalence of hemodialysis-related CHC than non-users (p<0.001). There was a significant (p<0.001), but weak (Cramer's V=0.180) correlation between hemodialysis-related CHC and aspirin exposure status.

Conclusion: These results indicated that regular use of aspirin might be linked to a lower risk of hemodialysis-related CHC. However, further prospective studies are required to confirm this association.

Keywords: Aspirin, chronic hepatitis C, hemodialysis

ÖZ

Amaç: Bu çalışmada hepatit C virüs (HCV) enfeksiyonu için yüksek risk gruplarından biri olan rutin hemodiyaliz hastalarında aspirin tedavisinin HCV enfeksiyonuna karşı korunmada etkili olup olmadığının değerlendirilmesi amaçlandı.

Gereç ve Yöntemler: Bu retrospektif kesitsel çalışma, Ocak 2017'de Hatay'da bulunan 4 özel hemodiyaliz merkezinde son dönem böbrek yetmezliği nedeniyle en az 3 ay süre ile rutin hemodiyaliz tedavisi almış olan 408 hastayı içermekte idi. Hastalar aspirin kullanım durumlarına göre iki gruba ayrıldı: İlk grup hemodiyaliz başlangıcından itibaren hiç aspirin kullanmamış 228 hastadan, ikinci grup ise hemodiyaliz başlangıcından itibaren düzenli aspirin kullanmakta olan 180 hastadan oluşmakta idi. Hemodiyaliz ilişkili kronik hepatit C (KHC) oranları gruplar arasında karşılaştırıldı. HBV ile enfekte, hemodiyaliz tedavisi öncesinde KHC tanısı almış olan, düzensiz aspirin kullanan hastalar çalışmaya dahil edilmedi.

Bulgular: Hemodiyaliz ilişkili KHC prevalansı toplam 408 hastada %3,9 idi. Düzenli aspirin kullanımı olan 180 hemodiyaliz hastasının hiçbirinde hemodiyaliz ilişkili KHC görülmedi. Düzenli aspirin kullanıcılarında hemodiyaliz ilişkili KHC prevalansı hiç aspirin kullanmamış hastalarla karşılaştırıldığında anlamlı oranda daha düşük bulundu (p<0,001). Aspirin kullanım durumu ile hemodiyaliz ilişkili KHC arasında anlamlı (p<0,001), ancak zayıf (Cramer's V=0,180) bir korelasyon bulunmakta idi.

Sonuç: Bu sonuçlar düzenli aspirin kullanımının hemodiyaliz ilişkili KHC riskinin azaltılmasında yararlı olabileceğini düşündürmektedir. Bununla birlikte, bu varsayımı doğrulamak için daha ileri prospektif çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Aspirin, kronik hepatit C, hemodiyaliz

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Introduction

Hepatitis C is a global health problem affecting an estimated 2.35% of the world population, and individuals undergoing maintenance hemodialysis (HD) are known to have a 5-fold greater risk of hepatitis C compared with the general population (1,2). Moreover, it has been reported that the presence of hepatitis C might be associated with increased mortality and graft rejection in end-stage renal disease patients undergoing HD or kidney transplantation (3).

Despite improvements in the treatment of chronic hepatitis C (CHC) in HD patients, this infection remains significant in HD patients as current treatments cannot prevent re-infections and there is currently no vaccine to prevent hepatitis C (4). Therefore, there is a need for alternative protective measures for the prevention of hepatitis C in HD patients.

Aspirin, also known as acetylsalicylic acid is a cyclooxygenase-1 (COX-1) and -2 (COX-2) inhibitor which has an antiviral effect on RNA viruses, including hepatitis C virus (HCV) (5,6). In recent years, *in vivo* and *in vitro* studies have shown that the use of aspirin increases the efficacy of standard antiviral therapy by suppressing HCV replication, slowing the progression of liver damage and preventing hepatocellular carcinoma development in CHC patients (7,8,9). Consistent with these data, Manning et al. (7) showed that successful antiviral therapy resulted in the normalisation of COX-2 over-expression which was induced by HCV infection. Claudin-1 is a tight junction protein which is highly expressed in the liver and known to be an essential factor for HCV entry (10). In a more recent study of the antiviral effect of aspirin, Yin et al. reported that aspirin inhibited the entry of HCV by decreasing the expression of claudin-1 (11).

According to the results of these studies, it was hypothesised that aspirin use may be effective in preventing HCV infections/re-infections in maintenance HD patients who have an increased risk for HCV infection. The aim of this study was therefore to investigate whether aspirin use is effective in the prevention of HCV infection in maintenance HD patients.

Materials and Methods

We conducted a cross-sectional retrospective study in January 2017 at four private HD units in Hatay, Turkey. A total of 408 patients with end-stage renal failure undergoing maintenance HD for at least three months were enrolled. Inclusion criteria for this study were (1) maintenance HD for at least three months. Exclusion criteria were (1) diagnosis of CHC before initiation of HD therapy, (2) being infected with hepatitis B (3) irregular aspirin use (<3 days per week), and (4) age <18 years. The participants were interviewed and the following data were obtained using a standardised questionnaire and were checked from medical records: presence of HBV infection, a history of drug abuse, blood transfusion(s) or renal transplantation, aspirin exposure information (dose, the frequency of use) and the date of initiation of HD. In Turkey, HD patients are examined routinely every three months for viral hepatitis and patients with positive anti-HCV are also tested for HCV RNA. Thus, serum anti-HCV and HCV RNA results since the initiation of HD were obtained from medical records retrospectively. The positivity of anti-HCV and HCV RNA for at least six months was defined as CHC. Patients diagnosed with CHC after initiation of HD therapy were defined as having HD-related CHC.

The patients were classified in two groups (regular aspirin users and non-users) based on aspirin exposure status since the initiation of HD. These exposure categories were selected according to prior studies showing that the antiviral effect of aspirin for HCV was time-dependent and was highest at 72 hours post-treatment (12). Regular aspirin use was defined as use of any dose of aspirin at least 3 times per week from the initiation of HD for at least 3 months. Patients, who reported no aspirin use, were defined as non-users. The presence of HD-related CHC, the duration of HD, the rate of patients with a history of blood transfusion or renal transplantation were compared between the two groups.

Low-dose aspirin was defined as a dose of 100-150 mg and high-dose aspirin was defined as a 300 mg dose.

To control microbial contamination of dialysis machines, hot-water (at 80 °C) rinsing was applied after each dialysis session and chemical disinfection was performed at the end of the day and at the end of the week. Disposable dialysis kits and needle sets were used and standard precautions, such as hand hygiene, personal protective equipment, and disinfection of equipments and surfaces, were followed. All patients positive for hepatitis B surface antigen and anti-HCV were dialyzed on dedicated dialysis machines in separate rooms. These standard precautions and practices were applied to control infection in each of four HD units.

Statistical Analysis

SPSS software package was used for statistical analyses (version 23.0, Chicago, IL, USA). Histograms, probability plots and the Kolmogorov-Smirnov/Shapiro-Wilk tests were used for testing of distribution normality. To compare variables between the two groups, the Mann-Whitney U test was used to compare non-normally distributed variables and chi-square test/Fisher's exact test (where appropriate) for categorical variables. The correlation between aspirin exposure status and the percentage of HD-related CHC were tested using the chi-square test of Independence. A p-value of less than 0.05 was considered statistically significant.

Results

The study included 228 non-users and 180 regular aspirin users. Of the 408 patients, 58.3% were female and 41.7% were male. The median age of the patients was 60.3 (18-95) years. The median duration of HD was 52 (3-336) months. In the total of 408 patients, the prevalence of HD-related CHC was 3.9%. The patients' characteristics are shown in Table 1.

None of the patients had a history of intravenous drug abuse or tattooing.

The most striking result to emerge from the data was that HD-related CHC infection was not seen in any of the 180 patients who had been receiving aspirin therapy regularly since the initiation of HD.

The chi-square test of independence showed a significant ($p<0.001$), but weak (Cramer's $V=0.180$) correlation between HD-related CHC and aspirin exposure status.

The proportion of patients with HD-related CHC was significantly lower in the regular aspirin user group than in the non-user group, although patients in the regular aspirin user group were older and

had a longer duration of HD (in months) than those in the non-user group. In addition, there was no significant difference in the proportion of patients with HD-related CHC between the groups when the patients are classified according to the duration of HD (in months) (p=0.179) (Table 2).

To analyze any association between aspirin dose and the risk of HD-related CHC, drug exposure data was obtained. Most patients (86.6%) were using low dose (100-150 mg) aspirin.

Discussion

In this study, it was investigated whether regular aspirin use was effective in preventing HCV infection in maintenance HD patients. The results indicate that regular use of aspirin may be associated with a decreased risk of HD-related CHC. Previous studies have reported that the expression of HCV proteins increase intracellular reactive oxygen species levels (13). Moreover, a number of authors have reported that antioxidants, including aspirin, modulate the oxidative stress induced by HCV at the same time by decreasing viral replication as well as decreasing viral protein expression. It has been suggested that the antiviral activity of aspirin might be mediated by the modulation of oxidative stress (14,15). Overall, there seems to be some evidence to suggest that aspirin has an antiviral effect against HCV. However, there are only limited data on the prophylactic effect of aspirin against HCV. These results therefore need to be interpreted with caution and large randomised controlled trials could provide more definitive evidence.

Although most studies in the field of the antiviral effect of aspirin have focused on controlling viral replication and protein expression, to the best of our knowledge, a pre-clinical study by Yin et al. is the only one which has directly investigated the potential prophylactic role of aspirin in HCV infection (11). The authors revealed that aspirin inhibited the entry of HCV by decreasing the expression of claudin-1, which is a tight junction protein that is highly expressed in the liver and an essential factor for HCV entry (10,11,14,16). This also concurs with previous reports, which showed that aspirin had an anti-HCV effect through down regulation of HCV protein expression (12,16). In the current study, the most important clinically relevant finding was that HD-related CHC infection was not seen in any of the patients who had been receiving aspirin therapy regularly since the initiation of HD. The present findings seem to be consistent with those of Yin and Zhang (11) and indicate that regularly taking aspirin every other day could protect against CHC infection in maintenance HD patients. This is also consistent with earlier observations, which showed that the antiviral effect of aspirin was still maximal 3 days after the last administration and the antiviral effect of aspirin was highest at 72 hours post-treatment, because we defined the regular aspirin use as any dose of aspirin ≥3 days per week in the current study (12). Nevertheless, because of the retrospective nature of the study and small number of HD-related CHC cases this result should be interpreted with caution. Further research on this topic is required to confirm these speculations.

COX-2 has been shown to be over-expressed in patients with CHC (7). Recent *in vitro* studies have speculated that COX-2 activity may be involved in aspirin-mediated down regulation of viral replication and protein expression (12,16). Even though aspirin has a very short half-life in plasma, aspirin irreversibly inactivates the COX activity of the platelets and the platelet reactivity has been seen to be normalized 96 hours after the cessation of a single oral dose of 100 mg aspirin (5,17). However, it is unclear whether the antiviral effect of aspirin also continues for 96 hours. Further experimental investigations are needed to determine when this antiviral activity of aspirin disappears after the last dose of aspirin intake in HD patients.

In an investigation of the anti-HCV effect of aspirin, the researchers found that aspirin had a dose-dependent antiviral effect (12). Therefore, to clarify any association between aspirin dose and the risk of HD-related CHC, drug exposure data was obtained.

Table 1. Characteristics of each group and statistical differences between groups

		Non-user group (n=228)	Regular aspirin user group (n=180)	p
Age, median (IQR)		60.5 (18-95)	63 (21-89)	0.005[a]
Gender	Male (%)	56.1	61.1	0.312[b]
	Female (%)	43.9	38.9	
Duration of hemodialysis (in months)		48 (3-336)	72 (3-168)	0.020[a]
Presence/proportion of hemodialysis related CHC, n (%)		16 (3)	0 (0)	<0.001[b]
Blood transfusion(s), n (%)	Yes	71 (31.1)	66 (36.7)	0.241[b]
	No	157 (68.9)	114 (63.3)	
Renal transplantation, n (%)	Yes	23 (10.1)	13 (7.2)	0.311[b]
	No	205 (89.9)	167 (92.8)	

[a]Mann-Whitney U test, [b]Chi-square test, IQR: Interquartile range, CHC: Chronic hepatitis C

Table 2. Comparison of the presence of hemodialysis related chronic hepatitis C between the groups according to the duration of hemodialysis

	Hemodialysis related CHC (+)		Hemodialysis related CHC (-)		p
	Regular aspirin users	Non-users	Regular aspirin users	Non-users	
Duration of hemodialysis ≤84 months	0	9	159	166	0.179[a]
Duration of hemodialysis >84 months	0	7	18	46	-

[a]Chi-square test, CHC: Chronic hepatitis C

However, probably because the HD population is at high risk of bleeding events, most patients were using low-dose aspirin (18). Overall, each dose category (low and high-dose) was too small to provide valid results. Therefore, it was not possible to investigate whether there was a relationship between the dose of aspirin and the risk of HD-related CHC. There is a need for further research on this topic to provide a better understanding of the optimal dose of aspirin to reduce the risk of CHC.

Study Limitations

Finally, a number of important limitations need to be considered. First, the major limitation was the small number of HD-related CHC cases. Secondly, there was no information about the total number of blood transfusion units since initiation of HD, which may have increased the risk of HD-related CHC infection. The retrospective nature of the study was another limitation. Despite these limitations, this research is the first clinical trial testing the hypothesis that aspirin has a prophylactic effect against CHC in a high-risk group, such as the group of HD patients included here. The results of this research provide further support for the hypothesis that the use of aspirin has an antiviral effect against HCV and reduces the risk of CHC.

Conclusion

Although the current study is based on a small sample of participants, the findings suggested that a regular every other day regimen of low-dose aspirin in maintenance HD patients might reduce the risk of CHC. Nevertheless, there is a need for further large-scale prospective studies to confirm these findings.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of Mustafa Kemal University, Faculty of Medicine, Hatay, Turkey (decision date: 09.02.2017, decision number: 12).

Informed Consent: Informed consent was not necessary because of the retrospective design of the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.B., Design: T.B., Y.Ö., S.İ.Ş., F.H.T., Data collection or Processing: T.B., Y.Ö., S.İ.Ş., F.H.T., Analysis or Interpretation: T.B., Y.Ö., S.İ.Ş., F.H.T., Literature Search: T.B., Writing: T.B.

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