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문헌의 질 평가 실습자료

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[실습자료1]

AMSTAR

질 문	판 단	판단근거
1. '사전에' 체계적 문헌고찰의 계획이 수립되었는가? 고찰 수행 전에 핵심질문과 포함기준이 확립되어야 한다.	□ 예 □ 아니오 □ 대답할 수 없음 □ 적용할 수 없음	
2. 문헌선택과 자료추출을 여러 명이 수행하였는가? 적어도 두 명의 연구자에 의해 독립적으로 문헌선택과 자료추출이 수행되어야 하고, 의견 불일치를 해소한 합의 과정이 제시되어야 한다.	□ 예 □ 아니오 □ 대답할 수 없음 □ 적용할 수 없음	
3. 포괄적인 문헌검색을 하였는가? 적어도 두 개의 전자 자료원을 이용하여 검색되어야 한다. 검색연도와 데이터베이스(예: Central, EMBASE, MEDLINE), 주제어(MeSH 제시 가능)가 기술되어야 하고, 실행 가능한 검색전략이 제시되어야 한다. 최신지견, 종설, 교과서, 특성화된 연구 등록원(specialized register) 검토, 해당분야 전문가 자문, 참고문헌 검토 등을 통해 검색이 보완하여야 한다.	□ 예 □ 아니오 □ 대답할 수 없음 □ 적용할 수 없음	
4. 포함기준에 출판상태(예: 회색문헌)가 사용되었는가? 출판여부에 관계없이 문헌이 검색되었는지, 출판상태와 언어 등에 따라 문헌을 배제했는지 여부가 기술되어야 한다.	□ 예 □ 아니오 □ 대답할 수 없음 □ 적용할 수 없음	
5. 포함 및 배제된 연구 목록이 제시되었는가? 포함 및 배제된 연구 목록이 제시되어야 한다.	□ 예 □ 아니오 □ 대답할 수 없음 □ 적용할 수 없음	
6. 포함된 연구의 특성이 제시되었는가? 개별연구의 연구대상, 중재, (중재)결과가 표 등의 형태로 제시되어야 한다. 분석된 연구의 특성(예: 연령, 인종, 성별, 사회경제적 상태, 질병상태, 이환기간, 중증도, 동반질환)이 제시되어야 한다.	□ 예 □ 아니오 □ 대답할 수 없음 □ 적용할 수 없음	
7. 포함된 연구의 질이 평가되고 기술되었는가? 사전에 계획된 평가 방법을 제시하여야 한다. 예를 들어 효과성 평가 연구에서는 무작위 위약대조 이중 눈가림 연구만을 포함시킬 수 있고 배정은폐를 포함기준으로 사용하기도 한다. 다른 연구 형태에는 특정 기준이 더 적합할 수 있다.	□ 예 □ 아니오 □ 대답할 수 없음 □ 적용할 수 없음	

질 문	판 단	판단근거
8. 포함된 연구의 질은 결론을 도출하는데 적절히 사용되었는가? 방법론적 엄격성과 질평가 결과가 자료분석, 결론도출 시 고려되었다.	□ 예 □ 아니오 □ 대답할 수 없음 □ 적용할 수 없음	
9. 개별연구의 결과를 결합한 방법이 적절하였는가? 연구들의 동질성을 평가하여 결과의 결합 가능성이 검정되어야 한다(예: 동질성에 대한 카이 제곱 검정, I^2). 이질성이 있다면 무작위 효과 모형(random effects model)을 사용하고 결과를 결합하는 것이 임상적으로 적절한 지 고려되어야 한다(예: 결합하는 것이 합리적인가?).	□ 예 □ 아니오 □ 대답할 수 없음 □ 적용할 수 없음	
10. 출판 비뚤림의 가능성을 평가하였는가? 출판 비뚤림의 가능성을 그래프(예: funnel plot 등) 또는 통계적 검정 결과(예: Egger 회귀검정)로 평가하여야 한다.	□ 예 □ 아니오 □ 대답할 수 없음 □ 적용할 수 없음	
11. 이해상충이 기술되었는가? 체계적 문헌고찰 및 포함된 연구들의 연구비 출처가 명확하게 제시되어야 한다.	□ 예 □ 아니오 □ 대답할 수 없음 □ 적용할 수 없음	

“대답할 수 없음”: 시행할 수 있으나 시행여부가 기술되지 않은 경우
“적용할 수 없음”: 시행할 수 없는 경우(예: 메타분석이 가능하지 않거나 저자에 의해 시도되지 않은 경우)

The Effect of Exercise on Prevention of the Common Cold: A Meta-Analysis of Randomized Controlled Trial Studies

Original Article

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Background: Because there is no specific treatment for the common cold, many previous studies have focused on prevention of the common cold. There were some studies reporting that regular, moderate-intensity exercise increases immunity and prevents the common cold. We conducted a meta-analysis to determine the effects of exercise on prevention of the common cold.

Methods: We searched MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), CINAHL for studies released through June 2013. We manually searched the references. Two authors independently extracted the data. To assess the risk of bias of included literature, Cochrane Collaboration's tool for assessing risk of bias was used. Review Manager ver. 5.2 (RevMan, Cochrane Collaboration) was used for statistical analysis.

Results: Four randomized controlled trials were identified. A total of 281 participants, 134 in the exercise group and 147 in the control group, were included. The effect of exercise on the prevention of the common cold had a relative risk (RR) of 0.73 (95% confidence interval [CI], 0.56 to 0.95; I^2 = 7%). The mean difference of mean illness days between exercise group and control group was -3.50 (95% CI, −6.06 to −0.94; I^2 = 93%). In the subgroup analysis, the RR of under 16 weeks exercise was 0.79 (95% CI, 0.58 to 1.08).

Conclusion: In this meta-analysis, regular, moderate-intensity exercise may have an effect on the prevention of the common cold. But numbers of included studies and participants were too small and quality of included studies was relatively poor. Subsequent well-designed studies with larger sample size are needed to clarify the association.

Keywords: Exercise; Common Cold; Meta-Analysis; Prevention

INTRODUCTION

The common cold is one of the most common diseases occurring among all age groups and is the primary cause of visits to doctors in developed countries. According to The National Institute of Allergy and Infectious Disease,[1] one billion common colds occur annually in the US and the average adult contracts two to four colds and children contract six to ten colds annually in the US. The socioeconomic cost of the common cold can be quite high ($5 billion per year in the US),[2] as it is the one of the

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most common causes of absenteeism from work and school. Viruses are the most common cause of colds. The most causative virus is the rhinovirus, which accounts for 40% of colds. Thus, the term 'common cold' does not refer to a single entity but to a group of diseases caused by numerous unrelated ethological agents,[3] so there is no specific treatment for the common cold besides symptomatic treatments. Therefore, prevention is the primary focus in managing the common cold. Hand-washing is the most effective and well-known preventive methods. A meta-analysis regarding the effects of probiotics on the prevention of the common cold[4] showed that probiotics may have a preventive effect against the common cold. In addition, a recent Cochrane review reported that routine vitamin C supplementation did not reduce the incidence of colds in the general population but that it may be useful for people exposed to brief periods of severe physical exercise.[3]

Some studies revealed that regular, moderate-intensity exercise affects the concentration of various cells impacting the body's immune system, especially immunoglobulin A (IgA) and natural killer (NK) cells.[5] In a randomized trial about the effects of moderate-intensity exercise on immune response,[6] 45 minutes of moderate-intensity brisk walking for 15 weeks was not associated with an improvement in lymphocyte function but was associated with a 20% increase in serum immunoglobulin including IgA, immunoglobulin M, and immunoglobulin G. Another study investigating the association between moderate-intensity exercise and upper respiratory tract infections (URTI)[7] reported that exercise reduced the number of days subjects suffered from URTI and the severity of their symptoms.

However, there has been no systematic review or meta-analysis of the effects of exercise on prevention of the common cold. Therefore, this study investigated the effects of regular, moderate-intensity exercise on the prevention of the common cold through a meta-analysis of randomized controlled trials.

METHODS

A meta-analysis of randomized controlled trials was performed to investigate the effects of regular, moderate-intensity aerobic exercise on the prevention of the common cold in the general population.

1. Inclusion Criteria

The systematic review included randomized controlled trials comparing differences in the incidence rate of common colds between a regular, moderate-intensity aerobic exercise group and a control group with no exercise. Regular exercise was defined as exercise performed more than 5 times per weeks, and moderate-intensity was defined as greater than 60% of maximal heart rate. Studies about lower respiratory tract diseases, such as pneumonia, tracheitis, bronchitis; studies about URTI other than the common cold, such as tonsillitis or otitis media; and studies about specific diseases, such as diabetes or hypertension, were all excluded.

2. Search Methods

The final search was performed on June, 2013. All searches were performed by professional librarians. There was no language restriction.

The searches were made on MEDLINE (PubMed), EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), and CINAHL. The keywords for MEDLINE and EMBASE search were as follows.

#1 Colds, Common OR Common Colds OR Cold, Common OR Coryza, Acute OR Acute Coryza OR Catarrh OR Catarrhs OR Infection, Respiratory Tract OR Respiratory Tract Infection OR Respiratory Infections OR Infections, Respiratory
Infections, Respiratory Tract OR Upper Respiratory Tract Infections OR Infections, Upper Respiratory Tract OR Upper Respiratory Infections OR Infections, Upper Respiratory OR Respiratory Infection, Upper OR Rhinoviruses OR Coryza Viruses
Coryza Virus OR Common Cold Virus OR Cold Virus, Common OR Cold Viruses, Common OR Common Cold Viruses

#2 Exercises OR Exercise, Physical OR Exercises, Physical OR Physical Exercise
Physical Exercises OR Exercise, Isometric OR Exercises, Isometric OR Isometric Exercises OR Isometric Exercise OR Warm-Up Exercise OR Exercise, Warm-Up OR Exercises, Warm-Up OR Warm Up Exercise OR Warm-Up Exercises OR Exercise, Aerobic OR Aerobic Exercises OR Exercises,

Aerobic OR Aerobic Exercise
#3 Controlled Clinical Trials, Randomized OR Clinical Trials, Randomized OR Trials, Randomized Clinical
#4 #1 AND #2 AND #3

In addition, an additional search was performed on the reference listed in each of the included studies.

3. Study Selection

Two independent authors reviewed the search results and selected studies satisfying the inclusion criteria; any disagreements between two authors were settled by discussion and consensus. If the two authors were unable to reach an agreement, a final decision was made by the third author.

4. Assessment of Risk of Bias

Quality assessment of the selected studies was performed using the Cochrane Collaboration's tool for assessing risk of bias.[8] Each item was classified as low risk, high risk, or unclear; low risk for low risk of bias, high risk for high risk of bias, and unclear for difficult to decide. The assessment was done by two independent authors, and any discrepancies between the two authors were resolved by discussion and consensus.

5. Data Extraction

Two independent authors independently carried out the data extraction using standard data extraction forms. Numbers and characteristics of participants, type and duration of exercise, control group details, follow-up period, and outcome variables were extracted. Discrepancies between two authors were resolved by discussion.

6. Statistical Analysis

Dichotomous data were presented as relative risk with 95% confidence intervals (CI), while continuous data were presented as mean difference. Review Manager ver. 5.2 (RevMan; Cochrane Collaboration, Oxford, UK)[9] was used to analyze the study results. The results were summarized through a forest plot, and a funnel plot was planned to check for publication bias if a number of the studies were to be over 10. I^2 is a form of statistics that quantifies inconsistency, and the I^2 test was applied to identify heterogeneity as well. I^2 ranges from 0% to 100%, and values between 0% and 40% were interpreted as unimportant heterogeneity, up to 60% as moderate heterogeneity, and over 60% as considerable heterogeneity. In the case of statistical homogeneity, a fixed-effects model was applied, whereas in the case of statistical heterogeneity, a random-effects model of meta-analysis was used. P-values lower than 0.05 were considered statistically significant. A subgroup analysis was conducted to evaluate the duration of exercise.

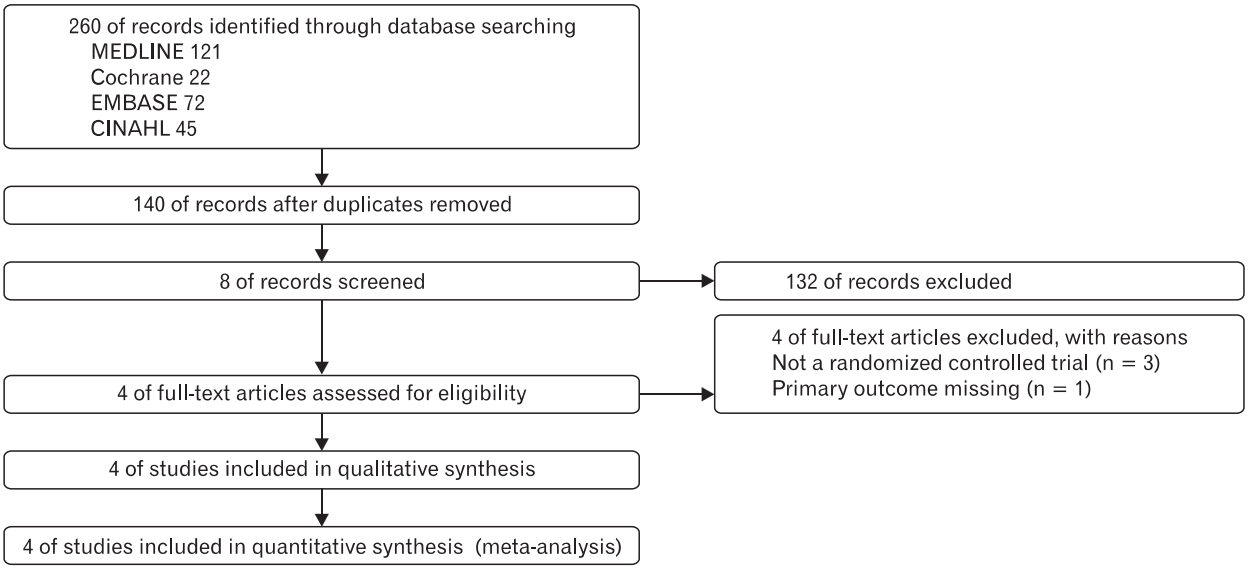

Figure 1. Figure 1. Flow sheet of study selection.

RESULTS

A total of 260 articles were retrieved from electronic searches including 121 articles from PubMed, 22 articles from Cochrane, 72 articles from EMBASE, and 45 articles from CINAHL. A total of 140 were screened and eight studies were found to be relevant to this study. Of these, five studies were selected during the full-text review process. One study[10] did not have the sufficient statistical data regarding the incidence rate of colds in the participants. The author of this study was contacted by e-mail, but did not reply. Thus four randomized controlled trials[11-14] were selected and included in the analysis (Figure 1). The characteristics of the included studies are shown in Table 1.[15] Ultimately, only three studies[11,13,14] out of four had sufficient data to extract the mean number of days participants were sick from the common cold.

A total of 281 participants were included; 134 participants were in the exercise group and 147 were in the control group. All studies were conducted in the US. One study[12] referred to the seasons during which trial was conducted while the remaining three did not. In three studies all participants were female, while in one study[13] 18% of participants were male. In one study[11] all participants were in their 30s, while in the other three studies all participants were in their 50s or older. In all included studies, monitoring of incidence and duration of common colds was done during the trial period. None was tested for long term effects. Two of the four studies[13] had measured the incidence and mean duration of upper respiratory infection during intervention period. In other study, participants were monitored two more months after intervention.

The risk of bias for the included studies was evaluated using the Cochrane Collaboration's tool for assessing risk of bias.[8] It was evaluated that items for appropriate random sequence generation and allocation concealment were low-risk in one study,[13] but deemed to be unclear in other three studies. Due to the nature of the studies, blinding of participants was not performed, though it was determined that the outcome was not likely to be influenced by a lack of blinding. Two studies[11,12] showed a high risk of bias regarding incomplete outcome data reporting. One study[13] showed a low risk of bias in selective reporting, and the others were unclear. Only one[13] study was evaluated as a relatively high

Table 1. Characteristics of included studies

Study	Study population	Age (y)	Exercise	Exercise duration	Compare	Outcome
Sloan et al.[14] (2013)	32 (exercise: 16, control: 16) healthy postmenopausal women	54.1 ± 5.3	Home-based walking program 5 days per week (30-minute brisk walking at a prescribed moderate aerobic exercise intensity corresponding to 75% of individual HRmax)	16 weeks	Continue with their usual daily physical activity	The type, frequency, duration and severity of any upper respiratory symptoms measured serum Ig A level
Barrett et al.[13] (2012)	98 (exercise: 47, control: 51) healthy adults (82% female, 94% white)	59.3 ± 6.6	8-week training in moderate-intensity sustained exercise	8 weeks	Observational control	Acute respiratory infection illness episode, severity health care visits and days of missed work
Chubak et al.[12] (2006)	115 (exercise: 53, control: 62) overweight and obese, sedentary, postmenopausal women in the Seattle area	Exercise: 60.5 ± 7.0 Control: 60.9 ± 6.8	45 minutes of moderate-intensity exercise 5 days per week (described in detail in Irwin et al.[15])	12 months	Once-weekly, 45-minute stretching session	No. of episodes of allergies, upper respiratory tract infections (colds and flu), and other infections
Nieman et al.[11] (1990)	36 (exercise: 18, control: 18) mildly obese, premenopausal sedentary women	Exercise: 36.0 ± 1.6 Control: 32.8 ± 1.4	Five 45-minute sessions/weeks, brisk walking at 60% heart rate reserve	15 weeks	Not to participate in any exercise outside of normal daily activity	Episode of upper respiratory infection Natural killer cell activity

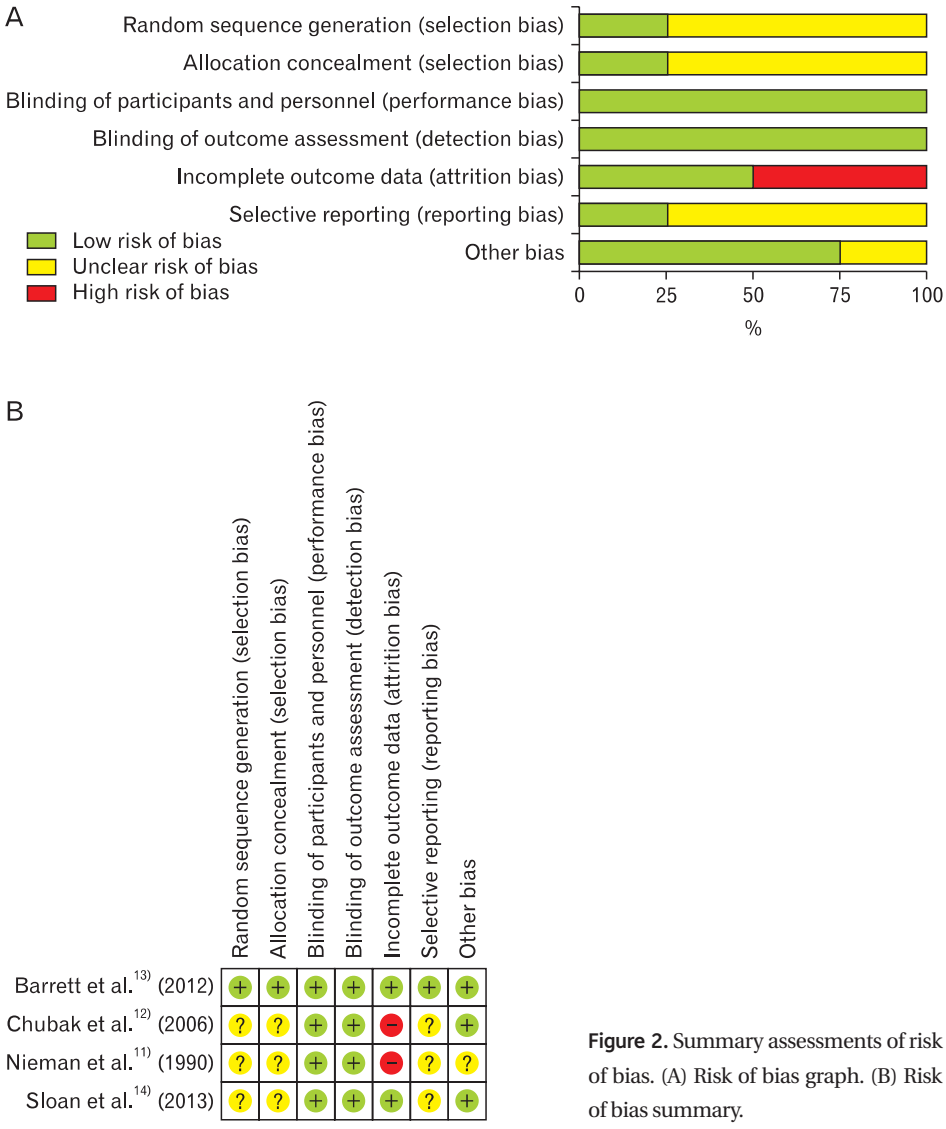

Figure 2. Summary assessments of risk of bias. (A) Risk of bias graph. (B) Risk of bias summary.

quality study; the other three studies did not provide enough data to assess the quality of the studies (Figure 2).

After analyzing all four studies, the relative risk of the common cold in exercise groups compared to the control group was 0.73 (95% CI, 0.56 to 0.95; $I^2 = 7\%$) (Figure 3). Three studies that reported mean illness days were analyzed as well. The mean difference between groups was −3.50 (95% CI, −6.06 to −0.94; $I^2 = 93\%$) (Figure 4). A subgroup analysis was conducted based on exercise duration. In the first subgroup with three studies[11,13,14] that had participants exercising for 16 weeks or less, the relative risk was 0.79 (95% CI, 0.58 to 1.08; $I^2 = 3\%$), but there was no statistically significant difference. In the second subgroup with one other study that had participants exercising for more than 16 weeks, the relative risk was 0.62 (95% CI, 0.46 to 0.89) (Figure 5). Because the number of included studies was less than 10, a funnel plot was not produced.

DISCUSSION

According to this meta-analysis of four studies about the effect of exercise on the prevention of the common cold, the relative risk of the common cold in exercise groups compared to the control group was 0.73 (95% CI, 0.56 to 0.95; $I^2 = 7\%$). The incidence rate of the common cold declined significantly in the exercise group, and there was no heterogeneity between studies. To identify the effect of exercise on the severity of the common cold, mean illness days as outcome variables was analyzed. Three

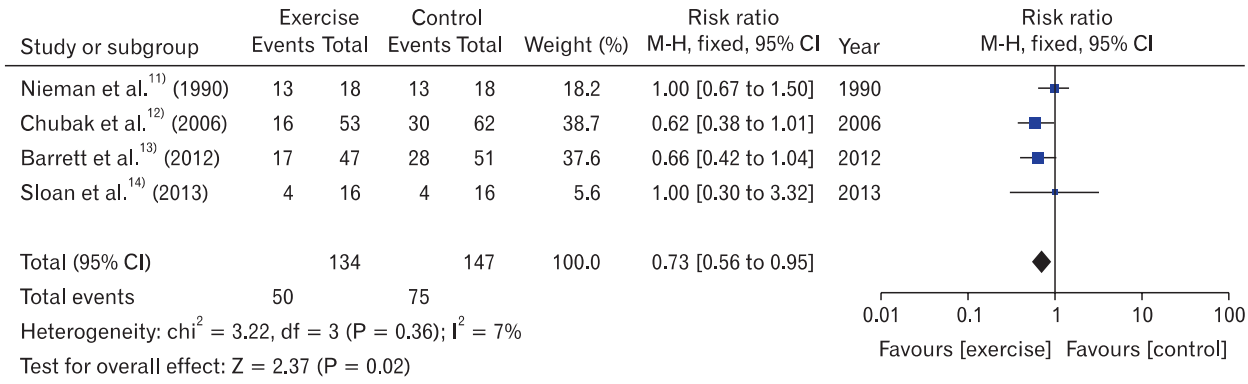

Figure 3. Meta-analysis: common cold incidence. CI: confidence interval.

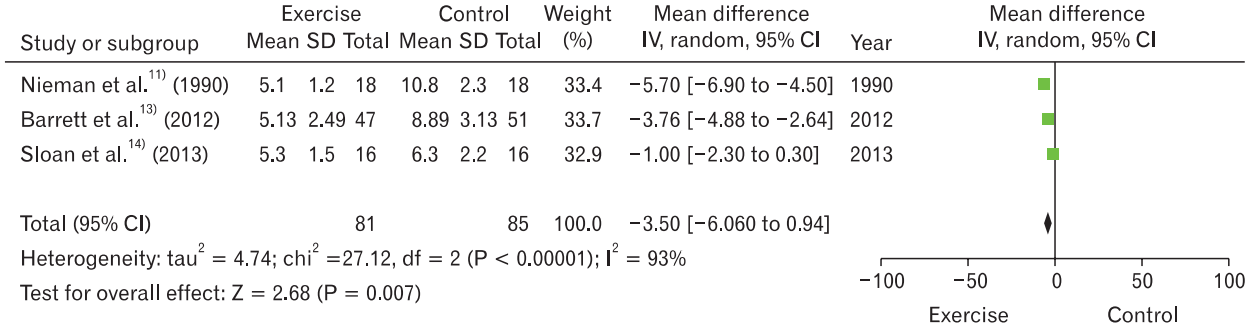

Figure 4. Meta-analysis: mean illness days. CI: confidence interval.

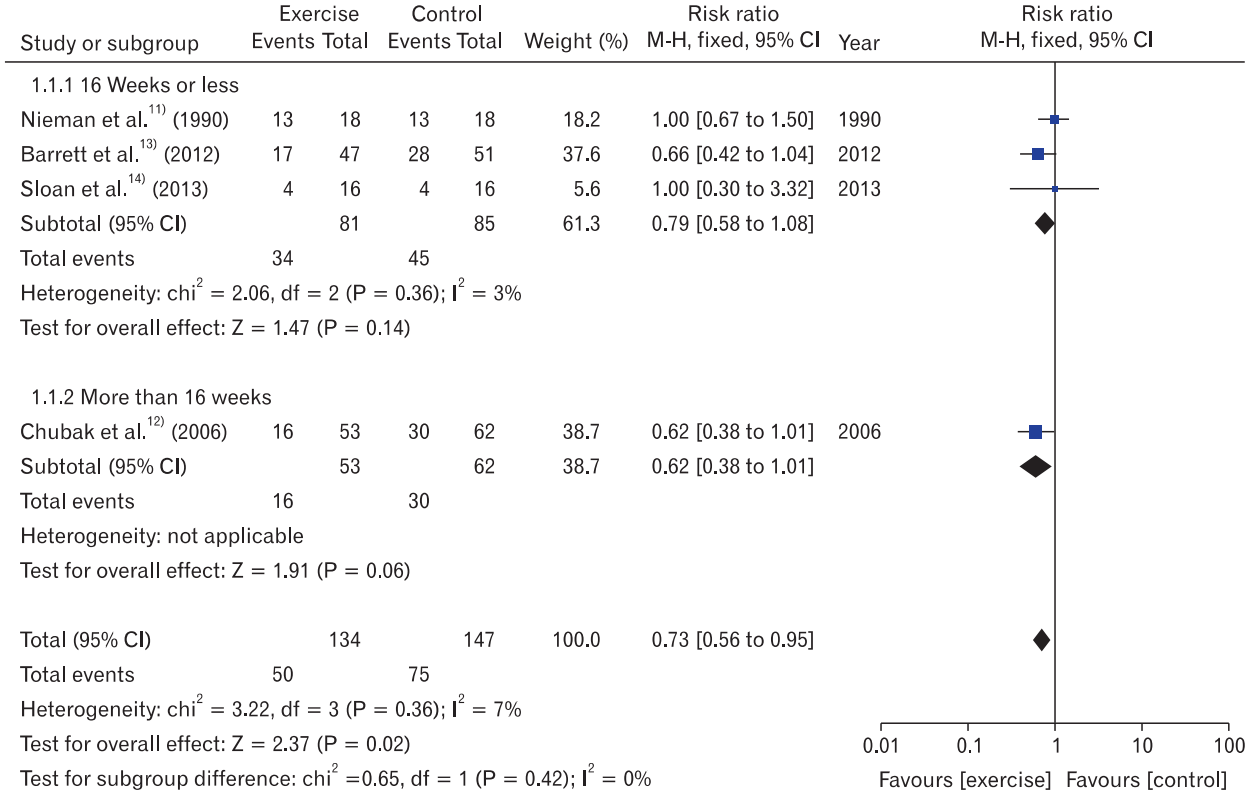

Figure 5. Subgroup-analysis: duration of exercise. CI: confidence interval.

studies had data outcome regarding mean illness days, and the mean difference was -3.50 (95% CI, −6.06 to −0.94; I^2 = 93%), with significant heterogeneity between studies.

In a subgroup analysis based on exercise duration, the relative risk of the subgroup with participants who exercised for 16 weeks or less was 0.79 (95% CI, 0.58 to 1.08; I^2 = 3%), but there was no statistically significant difference between groups. There was only one study with participants who exercised for more than 16 weeks exercise, and thus a subgroup comparison was not relevant. Because only four studies were included in this meta-analysis, it was not possible to draw a meaningful conclusion as to how exercise duration may help to prevent the common cold.

Several mechanisms have been proposed to explain the relationship between moderate excise training and risk of URTI. Barrett et al.[13] evaluated potential preventive effects of exercise on incidence, duration of URTI. They also analyzed interleukin-8, neutrophil count—biomarker of inflammation. But, between-group differences were not statistically significant for these biomarkers. A randomly controlled 15-week exercise training study was conducted to investigate the relationship between changes in NK cell number, activity, and URTI symptomatology. Nieman et al.[11] founded that exercise training did have a significant effect on NK cell activity especially during the initial 6-week period. Another study demonstrated an inverse relationship between salivary IgA concentration and risk of URTI.[14] From the results of these studies, the protective effects of regular, moderate-intensity aerobic exercise on colds may be partially explained by the immune status difference between the exercise group and control group. However the findings from these studies are inconsistent.[16]

This study has several limitations. First, because only four studies met the inclusion criteria, there were limitations regarding power and homogeneity among studies. In addition, each study had a small sample size, so the total number of participants in the meta-analysis was also small. Furthermore, most of the participants were women. Therefore, the results may not be applicable to men. The quality of the studies also could not be evaluated precisely because the included studies did not provide enough data for an accurate quality assessment. It was not possible to analyze how time of year affects incidence of the common cold, as only one study mentioned the time of year during which the trial was conducted, and even in that study there were no data about seasonal incidence rates of the common cold. Participant age was not taken into consideration in this meta-analysis, even though age greatly influences incidence of the common cold. Of the four studies included in this final analysis, participants in three of the studies were all in their 50s or older, while participants in one study were all in their 30s. No children were included in this meta-analysis.

In conclusion, the effects of exercise on the common cold were investigated through a meta-analysis of randomized controlled trials. The results suggest that regular, moderate-intensity aerobic exercise may have preventive effects on colds. However, the number of included studies and participants was too small, the quality of selected studies was relatively poor, and there was insufficient analysis of variables that may impact incidence of the common cold. Therefore, there was not enough information to draw a clear conclusion from this meta-analysis. Subsequent well-designed, large randomized controlled trials are needed to clarify the association between exercise and incidence of the common cold.

CONFLICT OF INTEREST

No potential conflict of interest related with this article was reported.

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[실습자료2]

ROB tools

영역	설명	비뚤임 위험	판단 근거(논문에서 그대로 인용함)
무작위 배정순서 생성	무작위 순서의 부적절한 생성에 따른 선택 비뚤임(중재 배정 비뚤임)	☐ 낮음 ☐ 높음 ☐ 불확실	
배정순서 은폐	부적절한 배정순서 은폐에 따른 선택 비뚤임(중재 배정 비뚤임)	☐ 낮음 ☐ 높음 ☐ 불확실	
연구 참여자, 연구자에 대한 눈가림	연구 참여자, 연구자가 배정된 중재를 알게 됨으로 인한 실행 비뚤임	☐ 낮음 ☐ 높음 ☐ 불확실	
결과 평가에 대한 눈가림	결과평가자가 배정된 중재를 알게 됨으로 인한 결과 확인 비뚤임	☐ 낮음 ☐ 높음 ☐ 불확실	
불완전한 결과 자료	불완전한 결과자료의 특성이나 처리로 인한 탈락 비뚤임	☐ 낮음 ☐ 높음 ☐ 불확실	
선택적 결과 보고	선택적 결과 보고로 인한 보고 비뚤임	☐ 낮음 ☐ 높음 ☐ 불확실	
타당도를 위협하는 다른 잠재적 비뚤임	다른 영역에서 평가하지 못한 문제점으로 인해 발생한 비뚤임	☐ 낮음 ☐ 높음 ☐ 불확실	

무작위 배정순서 생성

무작위 순서의 부적절한 생성에 따른 선택 비뚤임(중재 배정 비뚤임)

비뚤임 위험 '낮음' 기준	순서 생성에 무작위방법을 시행한 경우 예를 들어, • 난수표 이용 또는 컴퓨터를 이용한 난수 생성 • 동전던지기, 카드나 봉투섞기(꺼낸 카드는 다시 집어넣어야함), 주사위 던지기, 심지뽑기, *최소화법 등 사용. 그러나 '난수' 임이 보장되는 수행과정 확인할 수 있어야 적절한 방법으로 볼 수 있음. 예를 들어, 동전을 던져서 앞면이 나오면 뒷면이 나올 때 까지 다시 던지지 않았음을 확인할 수 있어야 함 (배정순서 은폐와 연결됨). *최소화법은 엄밀히 말해서 무작위로 순서가 배정 되는 것이라고 볼 수는 없으나 적절한 과정에 의해 수행된 최소화 법은 제대로 수행된 무작위배정으로 간주함.
비뚤임 위험 '높음' 기준	순서 생성에 무작위방법을 시행하지 않았거나 부적절한 방법을 사용한 경우 예를 들어, • 생년월일, 내원일 등의 규칙을 이용한 배정 • 환자 등록번호 또는 병록번호의 홀수 짝수 등 규칙을 이용한 배정 • 임상가의 판단에 따른 배정 • 환자의 선호도에 따른 배정 • 검사결과에 의한 배정 • 검사결과 순 또는 약제가 준비되는 순 등 이용가능 순에 의한 배정 • 배정자가 임의로 배정
비뚤임 위험 '불확실' 기준	무작위 배정순서 방법에 대한 비뚤임 위험이 '낮음', '높음' 중 어디에 해당하는지 불확실한 경우

배정순서 은폐

부적절한 배정순서 은폐에 따른 선택 비뚤임(중재 배정 비뚤임)

비뚤임 위험 '낮음' 기준	적절한 방법에 의해 배정순서가 은폐됨으로써 연구자가 배정내용을 알 수 없는 경우 예를 들어, • 독립적인 중앙 무작위배정 및 관리(웹기반, 전화, 제 3의 관리기관에 의한 무작위배정 통제 등) • 무작위배정순서에 의해 일련번호가 기록되어 있는 동일한 모양의 포장 사용 • 일련번호가 기록된 불투명하고 봉해진 봉투에 의한 배정순서 보관 및 개봉
비뚤임 위험 '높음' 기준	배정순서가 은폐될 수 있는 방법을 사용하지 않았거나 부적절한 방법의 사용에 의해 배정순서가 은폐되지 않은 경우 예를 들어, • 난수 또는 무작위배정순서가 기재된 표를 이용한 무작위 이행 • 밀봉되지 않거나 투명하거나 일련번호가 없는 등 안전장치가 없는 무작위배정 봉투를 사용 • 교대 혹은 순환법 등의 순서를 사용 • 생일, 병록번호 등을 이용
비뚤임 위험 '불확실' 기준	배정순서 은폐 방법에 대한 비뚤임 위험이 '낮음', '높음' 중 어디에 해당하는지 불확실한 경우, 예를 들어 봉투에 의해 배정되어 있다고 했으나 일련번호, 밀봉, 투명 여부에 대한 기술이 없을 때

연구 참여자, 연구자에 대한 눈가림

연구 참여자, 연구자가 배정된 중재를 알게 됨으로 인한 실행 비뚤임

비뚤임 위험 ‘낮음’ 기준	다음 중 한 가지 이상에 해당되는 경우 • 눈가림이 시행되지 않았거나 불완전하나, 눈가림이 (중재)결과에 영향을 미치지 않을 것으로 판단되는 경우 • 눈가림을 채택하여 수행하였고 연구 참여자와 연구자에 대한 눈가림이 깨지지 않았을 것으로 확신되는 경우
비뚤임 위험 ‘높음’ 기준	다음 중 한 가지 이상에 해당되는 경우 • 연구 참여자와 연구자에 대한 눈가림이 시도되었으나 눈가림이 유지되지 않았을 것으로 판단되고, 눈가림이 결과평가에 영향을 미칠 것으로 판단되는 경우 • 눈가림이 (중재)결과에 영향을 미칠 수 있는 경우임에도 눈가림을 시행하지 않았거나, 눈가림을 시도하였으나 방법이 부적절한 경우
비뚤임 위험 ‘불확실’ 기준	다음 중 한 가지 이상에 해당되는 경우 • 눈가림에 대한 비뚤임 위험이 ‘낮음’, ‘높음’ 중 어디에 해당하는지 불확실한 경우 • 연구에서 해당 결과를 다루지 않은 경우

결과평가에 대한 눈가림

결과평가자가 배정된 중재를 알게 됨으로 인한 결과 확인 비뚤임

비뚤임 위험 ‘낮음’ 기준	다음 중 한 가지 이상에 해당되는 경우 • 결과평가에 대한 눈가림을 채택하여 수행하였고 결과평가자에 대한 눈가림이 깨지지 않았을 것으로 확신되는 경우 • 눈가림이 시행되지 않았으나, 눈가림이 결과평가에 영향을 미치지 않을 것으로 판단되는 경우
비뚤임 위험 ‘높음’ 기준	다음 중 한 가지 이상에 해당되는 경우 • 눈가림이 결과평가에 영향을 미칠 수 있는 경우임에도 눈가림을 시행하지 않은 경우 • 결과평가자에 대한 눈가림이 시도되었으나 눈가림이 유지되지 않았을 것으로 판단되고, 눈가림이 결과평가에 영향을 미칠 것으로 판단되는 경우
비뚤임 위험 ‘불확실’ 기준	다음 중 한 가지 이상에 해당되는 경우 • 눈가림에 대한 비뚤임 위험이 ‘낮음’, ‘높음’ 중 어디에 해당하는지 불확실한 경우 • 연구에서 해당 결과를 다루지 않은 경우

불충분한 결과자료

불충분한 결과자료의 특성이나 처리로 인한 탈락 비뚤임

비뚤임 위험 ‘낮음’ 기준	다음 중 한 가지 이상에 해당되는 경우 • 결측치가 없는 경우 • 결측치가 결과에 영향을 미치지 않는 경우(생존분석에서는 결측이 절단값으로 다루어짐) • 결측치가 중재군 간에 유사하게 발생하고 결측치가 발생한 원인도 유사함 • 이분형 변수의 경우 결측치 분율이 관찰발생위험을 비추어볼 때 중재효과 추정에 임상적으로 유의한 차이를 낼 것으로 보이지 않는 경우 • 연속형 변수의 경우 결측값들로부터 예견되는 중재효과의 크기가 관찰된 효과의 크기 추정에 임상적으로 유의한 영향을 미칠 것으로 보이지 않는 경우 • 적절한 통계적 방법을 사용하여 결측치를 대체한 경우

비뚤임 위험 '높음' 기준	다음 중 한 가지 이상에 해당되는 경우 • 상당수의 결측치가 존재하고 결측치의 원인이 실제 결과에 영향을 미칠 수 있는 경우 - 중재군 간의 불균형한 결측치 수 차이 자체 또는 결측이 생긴 이유가 결과에 비뚤임을 초래할 수 있는 경우 • 이분형 변수의 경우 결측치 분율이 결과변수의 관찰발생위험에 비추어 상당 수여서 중재효과 추정에 임상적으로 유의한 차이를 낼 것으로 보이지 않는 경우 • 연속형 변수의 경우, 결측 결과로부터 예견되는 군간 중재효과 차이가 (평균의 차이 혹은 표준화 평균의 차이)가 효과크기 추정결과에 임상적으로 유의한 비뚤임을 초래하기에 충분한 경우 • 무작위 배정된 중재를 받지 않은 사람이 상당수 임에도 중재 받은 대로만 분석을 수행하여(per-procotol analysis) 결과자료를 제시한 경우 • 부적절한 방법으로 결측치를 대체한 경우
비뚤임 위험 '불확실' 기준	다음 중 한 가지 이상에 해당되는 경우 • 배제/탈락에 대한 보고가 불충분한 경우(예, 무작위수 언급 없음, 결측 이유에 대한 언급 없음) • 연구에서 해당 결과를 다루지 않은 경우
선택적 보고 선택적 결과 보고로 인한 보고 비뚤임	
비뚤임 위험 '낮음' 기준	다음 중 한 가지 이상에 해당되는 경우 • 프로토콜이 존재하여 연구에서 사전에 정의해놓은 일차, 이차 (중재)결과들의 정의 및 분석이 사전에 정해진 방법대로 다루어졌음을 확인할 수 있는 경우 • 프로토콜은 없지만 사전에 계획된 것을 포함하여 예상되는 모든 결과를 보고하고 있는 경우
비뚤임 위험 '높음' 기준	다음 중 한 가지 이상에 해당되는 경우 • 사전에 정해진 (중재)결과이었음에도 결과가 보고되지 않은 것이 있는 경우 • 보고된 결과 중 사전에 정한대로 방법으로 측정하거나 분석하지 않은 경우 또는 사전에 정하지 않은 (중재)결과를 보고하는 경우 (이런 분석과 보고가 이루어진 데 대한 명백한 이유와 설명 -예를 들어, 예상치 못한 부작용 등- 이 있는 경우는 예외) • 불완전한 결과보고로 인해 메타분석에 포함시킬 수 없는 경우 • 현 연구에서 당연히 분석되었을 것으로 예상되는 핵심결과에 대한 보고가 없는 경우
비뚤임 위험 '불확실' 기준	'높음', '낮음'에 대한 판단을 위한 정보가 충분하지 않은 경우(대다수의 연구들이 이 범주에 포함될 가능성이 있음)
그 외 비뚤임 다른 영역에서 평가하지 못한 문제점으로 인해 발생한 비뚤임	
비뚤임 위험 '낮음' 기준	그 외 비뚤임이 없는 것으로 보임
비뚤임 위험 '높음' 기준	추가 비뚤임의 위험이 있는 것으로 판단 예를 들어, • 특정 연구 설계와 관련된 잠재적 비뚤임 위험이 있음 • 연구수행에 부정이 있었다는 주장이 제기된 바 있음 • 기타 다른 문제점을 가지고 있음
비뚤임 위험 '불확실' 기준	추가 비뚤임 가능성에 대한 여지가 있으나 비뚤임의 위험이 어느 정도일지 평가할만한 충분한 정보나 근거가 없는 경우

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Effect of Hydroxychloroquine Treatment on Dry Eyes in Subjects with Primary Sjögren's Syndrome: A Double-Blind Randomized Control Study

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The effect of hydroxychloroquine (HCQ) on dry eye has not been fully determined. This study aimed to compare the 12-week efficacy of HCQ medication with that of a placebo in the management of dry eye in primary Sjögren's syndrome (pSS). A double-blind, randomized control study was conducted in 39 pSS subjects from May 2011 through August 2013. pSS was diagnosed based on the classification criteria of the American-European Consensus Group. Subjects received 300 mg of HCQ or placebo once daily for 12 weeks and were evaluated at baseline, 6 and 12 weeks, with a re-visit at 16 weeks after drug discontinuance. The fluorescein staining score, Schirmer test score, tear film break-up time (TBUT), and ocular surface disease index (OSDI) were measured, and tears and blood were collected for ESR, IL-6, IL-17, B-cell activating factor (BAFF), and Th17 cell analysis. Color testing was performed and the fundus was examined to monitor HCQ complications. Twenty-six subjects completed the follow-up. The fluorescein staining score and Schirmer test score did not differ significantly. The OSDI improved with medication in the HCQ group but was not significantly different between the groups. TBUT, serum IL-6, ESR, serum and tear BAFF, and the proportion of Th17 cells did not change in either group. HCQ at 300 mg daily for 12 weeks has no apparent clinical benefit for dry eye and systemic inflammation in pSS (ClinicalTrials.gov. NCT01601028).

Keywords: Hydroxychloroquine; Dry Eye Syndromes; Sjögren's Syndrome; Double-Blind Method; Prospective Studies

INTRODUCTION

Sjögren's syndrome (SS) is a systemic autoimmune disease involving the lacrimal and salivary glands with resultant keratoconjunctivitis sicca and xerostomia, and lymphocytic infiltration of exocrine glands and epithelium is a common pathological finding (1). SS is one of the most prevalent rheumatologic diseases and has female predominance (2). Approximately one-third of SS patients have extraglandular systemic involvement, and SS patients with or without other autoimmune rheumatic disease are defined as having secondary SS (sSS) or primary SS (pSS), respectively. In 2002, the American-European Consensus Group defined the rules for classifying pSS and sSS mainly using a serologic marker (the anti-Ro/La antibody level) and the histopathology of the salivary glands (3).

Conjunctival and corneal staining tests, the Schirmer test, and symptoms are worse in patients with than without SS among aqueous-deficient dry eye patients (4). Conjunctivocorneal epithelial disintegration results in blurred vision, severe discomfort, and increased risk of infection. Therefore, severe dry eye in SS patients results in an ongoing poor quality of life.

The first treatment option for dry eye symptoms is topical drugs such as artificial tears or cyclosporine A (2,5). However, topical treatment is not usually sufficient for severe dry eye in pSS patients. Because inflammatory markers are increased in SS patients, most systemic medications are aimed at immunologic pathways (6). Antimalarial drugs are frequently prescribed to SS patients as non-specific blockers of toll-like receptor 9/7 (TLR9/TLR7), but their effectiveness in dry eye is controversial. Improvement in levels of immunologic markers such as IL-6, the erythrocyte sedimentation rate (ESR), C reactive protein (CRP), and B-cell activating factor (BAFF), in addition to sicca signs such as the tear break-up test (TBUT) and Schirmer test, has been reported in a few studies after oral management with hydroxychloroquine (HCQ) (7,8). However, only two double-blind randomized trials for pSS have been performed so far, which demonstrated that HCQ treatment does not have clinical benefits for pSS patients (9,10).

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Therefore, to evaluate the effect of oral HCQ treatment in dry eye in pSS, we conducted a prospective randomized, double-blind trial comparing the HCQ treatment with a placebo control.

MATERIALS AND METHODS

Patients

This randomized controlled trial was conducted at the Department of Ophthalmology at Seoul National University Hospital from May 2011 through August 2013. Inclusion criteria were 1) adult (aged > 18 years) patients with pSS diagnosed on the basis of the 2002 American-European Consensus Group (AECG) criteria (3) and 2) subjects who had the ability to give informed, dated, and signed consent before the beginning of any proceedings related to the trial.

Exclusion criteria were 1) previous treatment with HCQ with insufficient discontinuation time (3 weeks) after discontinuation of treatment; 2) known cardiac disease, respiratory disease, renal disease, or gastrointestinal disease (except gastrooesophageal reflux disease); 3) diabetes mellitus; 4) psoriasis; 5) known drug allergy or hypersensitivity; 6) previous or ongoing treatment with any other drugs (including topical drugs) that may affect the lacrimal system with insufficient washout time after discontinuance of treatment (e.g., SSRI, anti-histamines, and pilocarpine); 7) closed-angle glaucoma; 8) previous intraocular surgery; 9) macular disease; 10) previous or ongoing treatment with drugs that may have an effect on the macula; 11) pregnancy; and 12) planning pregnancy.

A double-blind, randomized control study was conducted in 39 subjects of 153 pSS subjects for 2 years. We were unable to recruit the intended 60 subjects within the 2-year recruitment period because many subjects with pSS did not meet our inclusion criteria. Most of the subjects did not wish to discontinue previous oral drugs because of severe systemic symptoms. During the study period, 153 pSS patients visited our clinic. Among them, only 67 subjects met all eligibility criteria. Twenty-eight subjects declined to participate in the trial, and 39 subjects were finally enrolled in this study (Fig. 1).

During the study period of 2 years, a new criterion for SS was reported (11). Because this study was designed before the recent criterion was published, we enrolled subjects according to the previous criteria of the American-European Consensus Group to maintain consistency of the subject population.

Treatment protocol

All subjects underwent initial medical and ophthalmologic history taking and physical examination at the baseline visit (week 0). The study eye was selected as the eye that showed the higher corneal fluorescein staining score (on a scale of 0-15; National Eye Institute scale [12]) at the baseline visit. Subjects were randomly assigned to the HCQ or placebo group by using sealed randomization envelopes. The placebo was manufactured to be identical in appearance to the active drug, and all tablets were kindly supplied by Kyung Poong Pharma Co. Both the investigators and subjects were blinded to the treatment assignments. The subjects were instructed to take two tablets (one 200 mg tablet and one 100 mg tablet) of the study medication, giving a total of 300 mg, once daily (qd) for 12 weeks and to report missed doses and adverse events. All subjects used Hyalein Mini ophthalmic solution 0.1% (0.1% hyaluronic acid; Santen, Osaka, Japan) six times per day. Other topical medications were restricted. Participants returned to the study site at 6 and 12 weeks for efficacy and safety evaluations. After 12 weeks, oral HCQ and placebo medication were withheld, whereas topical medication was continued in all subjects for 4 weeks. The subjects revisited the clinic at 16 weeks. At each visit, the visual acuity, corneal fluorescein staining score using NEI grading, Schirmer test score without anesthesia, tear film break-up time (TBUT), Har-

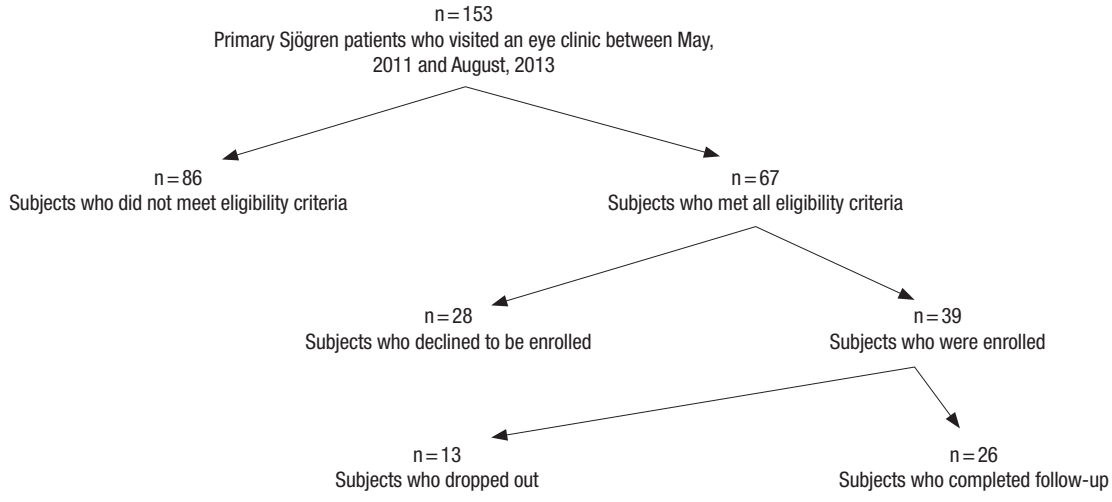

Fig. 1. A double-blind randomized control study was conducted in 39 subjects of 153 pSS subjects who visited an eye clinic from May 2011 through August 2013.

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dy Rand and Rittler (HRR) color vision test, and fundus examination were performed. Schirmer test was done with a Schirmer strips (Eagle Vision, Memphis, TN, USA) for five minutes without topical anesthesia. TBUT was measured after instillation of one drop of 0.25% fluorescein dye into the conjunctival sac, and the subjects were asked to blink several times. Subjective assessment was performed using the ocular surface disease index questionnaire (OSDI) (13). The OSDI questionnaire was translated to Korean from the original English. Tear and blood samples were collected for measurement of IL-6, BAFF, ESR, and IL-17 levels in addition to Th17 cells that secrete IL-17.

Tear sample collection

Tear fluid was obtained from the patients at the same time at baseline and at 6, 12, and 16 weeks. Tears were collected from the medial and lateral canthus. Topical anesthesia was not used. To minimize ocular surface irritation, we obtained tear samples by using a Merocel sponge (PVA 0525; Oasis, Glendora, CA, USA) (14). After collection, the sponge was inserted into a 0.5-mL tube (Eppendorf, Fremont, CA, USA) and the tear fluid was subsequently recovered by centrifugation at 10,000 rpm for 10 minutes.

Measurement of tear and serum cytokine profiles

ESR was measured by conventional methods in our hospital laboratory on the day of the patient visit. For BAFF and IL-6, 0.5 mL of plasma was separated from heparinized peripheral blood and stored together with the tear sample at -70°C until further examination.

The concentrations of BAFF, IL-6, and IL-17A were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. ELISA using serum was performed in duplicate to ensure the reproducibility of the data. However, ELISA using tears was performed only once because of the lack of a sufficient amount of tears. Tear samples were diluted 1:12.5 and 1:100 for BAFF and IL-17, respectively.

The concentration ranges used for the standard curve were 62.5-4,000 pg/mL, 9.38-600 pg/mL, and 15.6-1,000 pg/mL of human recombinant BAFF, IL-6, and IL-17, respectively (R&D Systems).

Flow cytometric analysis for Th17 cells

Flow cytometry was also performed on the same day as the patient visit. Peripheral blood mononuclear cells (PBMCs) were

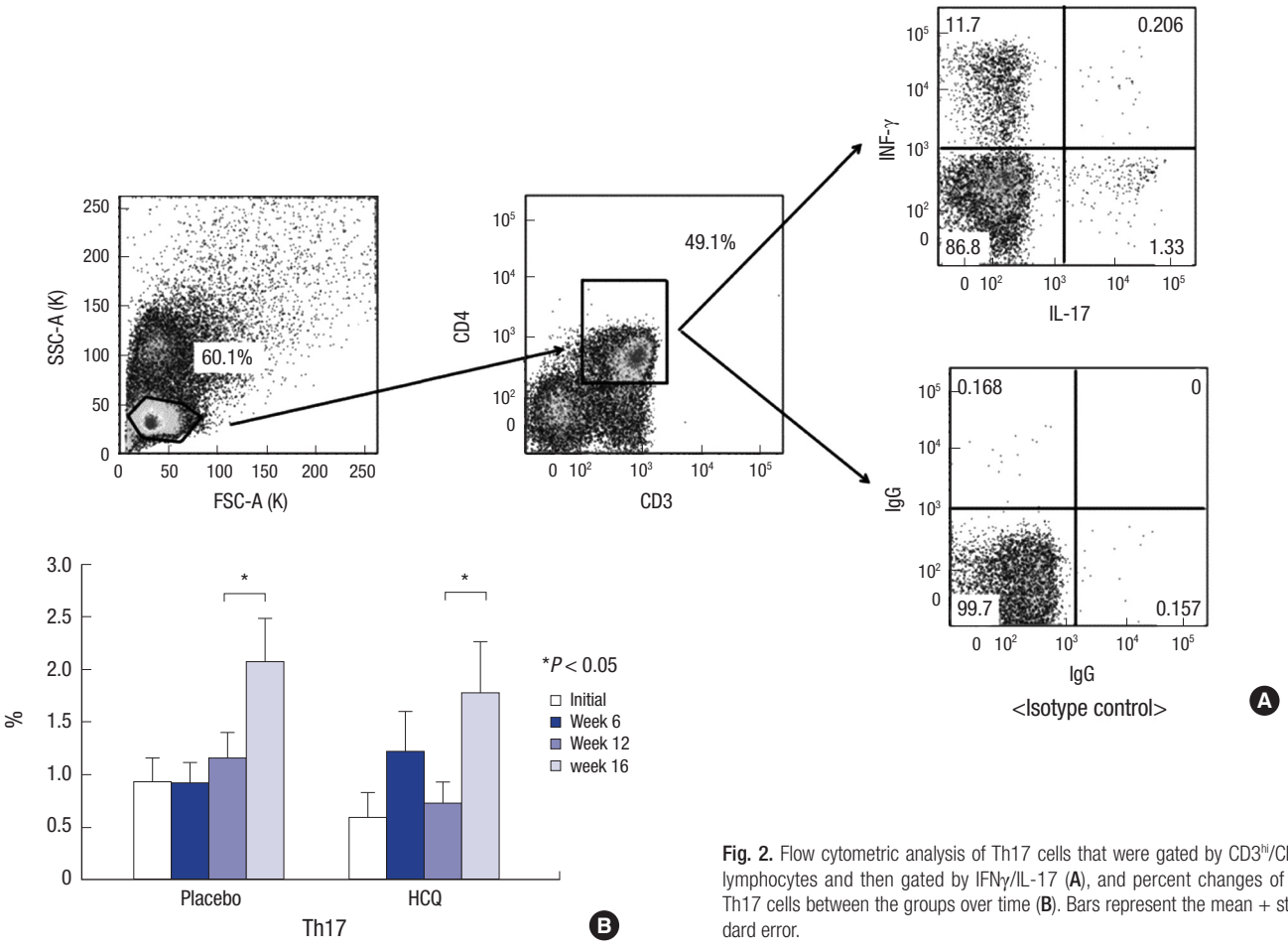

Fig. 2. Flow cytometric analysis of Th17 cells that were gated by $CD3^{hi}/CD4^{hi}$ lymphocytes and then gated by IFNγ/IL-17 (**A**), and percent changes of the Th17 cells between the groups over time (**B**). Bars represent the mean + standard error.

isolated from whole blood by density gradient centrifugation (BIOCHROM Inc., Cambridge, UK). PBMCs were stimulated for 6 hours with 50 ng/mL 1-phorbol-12-myristate-13-acetate (PMA; Sigma-Aldrich, St Louis, MO, USA) and 1 μg/mL ionomycin (Sigma-Aldrich) in the presence of brefeldin A (BD Bioscience, San Jose, CA, USA) for the final 4 hours. The cells were fixed and permeabilized using a BD Cytofix/Cytoperm kit (BD Bioscience). The fixed cells were stained with anti-CD3-APC-Cy7 and anti-CD4-FITC for 30 minutes at 4°C for initial surface staining. For intracellular staining, the cells were incubated with anti-IFN-γ-PE-Cy7 (all from BD Bioscience) and anti-IL-17A-PE (eBioscience, SanDiego, CA, USA) mAb for 60 minutes at 4°C. Flow cytometric analysis was performed using a FACS BD LSR II (BD Bioscience), and the data were analyzed by FlowJo (Treestar, Ashland, OR, USA). Isotype mouse Ig G1-PE-Cy7 (eBioscience) and mouse Ig G1-PE (eBioscience) were used as controls.

After four-color compensation, lymphocytes were gated in the forward scatter (FSC)/side scatter (SSC) gate, and CD4 T cells were then gated by $CD3^{hi}$ and $CD4^{hi}$ expression; finally, the cells were gated by IFNγ and IL17 expression (Fig. 2). The percentage of Th17 cells was calculated by summing the amounts of $IL17^{hi}$ cells and $IFN\gamma/IL17^{double\ hi}$ cells.

Sample size calculations

A previous literature review on SS did not include results for the tear IL-17 concentration after HCQ medication. The tear IL-17 concentration of SS patients was 504.91 pg/mL and 352.45 pg/mL in the total and mild keratoconjunctivitis sicca groups, respectively, in this previous report (15). We assumed that the tear IL-17 concentration after HCQ medication would be similar to that of the mild keratoconjunctivitis sicca group from the previous study. A sample size of 60 patients (30 in each group) was needed to detect a significant difference between 504.91 ± 136.38 pg/mL and 352.45 ± 136.38 pg/mL in the 2 groups using a 2-sided test with a power of 80% and the significance level controlled at 5%. The Medical Research Collaborating Center of Seoul National University Hospital assisted in the sample size calculation.

Statistical analysis

Primary analyses of the data were based on the per-protocol population, which included all participants who took the 12-week course of study medication. Statistical analyses were performed using PASW software for Windows (v. 19.0; SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant. The Shapiro-Wilk test was applied to evaluate data normality. The two groups were compared with Student's *t*-test (two-tailed) of the Mann-Whitney U-test depending on normality. To compare changes within groups at different time intervals, a paired sample *t*-test and Wilcoxon signed-rank test were used depending on normality. Differences between the HCQ and placebo groups during the course of the treatment were analyzed by repeated-measures ANOVA and linear mixed models when data were missing.

Ethics statement

Written informed consent was obtained from all subjects, and the study was granted ethical approval by the institutional review board of Seoul National University Hospital (IRB number: H-1104-083-359). This study was conducted in accordance with the tenets of the Declaration of Helsinki. This study was registered with ClinicalTrials.gov (Identifier: NCT01601028).

RESULTS

General complications of the subjects and characteristics of drop-out subjects

Of the 39 recruited subjects, 26 were treated per protocol. Thirteen subjects including five in the placebo group and nine in the HCQ group did not complete the study. The initial age, ESR, TBUT, Schirmer score, corneal staining score, and OSDI were not significantly different between complete and incomplete groups. In the placebo group, three subjects did not keep their follow-up appointments and were unavailable to be reached, one subject suffered dyspepsia, and one subject was found to be unsatisfactory for enrolment. In the HCQ group, two subjects did not keep their follow-up appointments and were unable to be contacted, three subjects suffered dyspepsia, one subject did not have the time for follow-up examinations, two subjects were found deficient with regard to the enrollment criteria, and one subject developed incidental subretinal hemorrhage because of occult myopic choroidal neovascularisation (CNV) with accompanying impaired vision 3 days after medication, which was not regarded as a drug-related complication. There were no other drug-related side effects with regard to ocular complications (optic neuropathy or maculopathy) or general subjective complications except dyspepsia (10.3%).

Characteristics of the study population at baseline

Of the 26 pSS subjects analyzed, 11 subjects were randomly assigned to receive HCQ and the remaining 15 subjects were randomly assigned to receive the placebo. All subjects were women with a mean age of 56.85 years (range, 35-74). The mean (range) age was 55.0 (35-73) years in the placebo group and 59.4 (44-74) years in the HCQ group, which was not significantly different ($P = 0.263$, independent *t*-test). The initial results for ESR, serum IL-6, BAFF, Th17, tear BAFF, TBUT, Schirmer score, corneal staining score, and OSDI are shown in Table 1. There were no significant differences between the groups for all of the demographic factors and parameters.

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Table 1. Demographics and initial characteristics of the subjects

Parameters	Total (n = 26)	Placebo (n = 15)	HCQ (n = 11)	*P*
Age, yr	56.85 ± 9.66	55.0 ± 9.72	59.4 ± 9.42	0.263*
ESR, mm/hr	23.42 ± 14.78	19.67 ± 12.08	28.55 ± 17.08	0.133*
Serum IL-6, pg/mL	4.31 ± 7.45	4.20 ± 4.90	4.45 ± 10.25	0.134†
Th17, %	0.80 ± 0.74	0.93 ± 0.80	0.61 ± 0.70	0.340†
Serum BAFF, pg/mL	2,300 ± 859	2,327 ± 1,028	2,261 ± 601	0.878†
Tear BAFF, pg/mL	384 ± 220	436 ± 328	417 ± 184	1.000†
TBUT, sec	2.52 ± 0.85	2.23 ± 0.63	2.78 ± 1.09	0.439†
Schirmer, mm	4.12 ± 3.90	4.00 ± 3.07	4.27 ± 4.98	0.878†
Corneal staining score	3.27 ± 1.71	3.33 ± 1.54	3.18 ± 1.99	0.829*
OSDI	47.92 ± 28.30	43.40 ± 27.33	54.08 ± 29.74	0.352*

HCQ, hydroxychloroquine; ESR, erythrocyte sedimentation rate; IL, interleukin; Th17, T helper 17 cells; BAFF, B-cell activating factor; TBUT, tear film break-up time; OSDI, ocular surface disease index.
*Independent *t*-test; †Mann-Whitney U-test.

Table 2. Changes of the parameters between the HCQ and placebo groups during follow-up

Parameters	Baseline	Week 6	Week 12	Week 16	*P* (baseline-week 12)	*P* (week 12 vs. 16)
ESR, mm/hr					0.571*	0.620*
Placebo	19.67 ± 12.08	20.13 ± 8.12	21.40 ± 11.38	20.20 ± 12.52		
HCQ	28.55 ± 17.08	27.36 ± 18.49	24.91 ± 20.94	21.09 ± 12.09		
IL-6, pg/mL					0.451†	0.991*
Placebo	4.20 ± 4.90	5.31 ± 8.35	5.01 ± 6.83	5.83 ± 8.37		
HCQ	4.45 ± 10.25	4.77 ± 9.21	5.37 ± 10.32	6.34 ± 14.14		
Th17, %					0.199†	0.566*
Placebo	0.93 ± 0.80	1.05 ± 0.73	1.14 ± 0.98	2.07 ± 1.48		
HCQ	0.61 ± 0.70	1.05 ± 1.32	0.91 ± 0.77	1.79 ± 1.65		
Serum BAFF, pg/mL					0.340†	NA
Placebo	2,327 ± 1,028	2,513 ± 1,903	2,480 ± 1,410	NA		
HCQ	2,261 ± 601	2,071 ± 492	2,116 ± 711			
Tear BAFF, pg/mL					0.723†	NA
Placebo	436 ± 328	141 ± 56	427 ± 256	NA		
HCQ	417 ± 184	1,094 ± 1,418	1,113 ± 1,466			
TBUT, sec					0.125†	0.746*
Placebo	2.23 ± 0.63	2.60 ± 0.83	2.87 ± 0.99	2.57 ± 0.76		
HCQ	2.78 ± 1.09	2.60 ± 0.84	2.45 ± 0.52	2.18 ± 0.40		
Schirmer, mm					0.136†	0.958*
Placebo	4.00 ± 3.07	4.33 ± 4.22	3.20 ± 2.68	3.43 ± 2.14		
HCQ	4.27 ± 4.98	2.50 ± 1.08	2.82 ± 2.40	2.91 ± 2.26		
Corneal staining score					0.128†	0.524*
Placebo	3.33 ± 1.54	3.20 ± 2.01	3.67 ± 1.54	4.07 ± 2.09		
HCQ	3.18 ± 1.99	3.10 ± 2.18	2.54 ± 2.16	2.54 ± 2.42		
OSDI					0.209*	0.292*
Placebo	43.40 ± 27.33	32.45 ± 19.21	30.47 ± 23.47	30.92 ± 28.17		
HCQ	54.08 ± 29.74	30.37 ± 28.63	22.88 ± 21.51	27.75 ± 21.73		

HCQ, hydroxychloroquine; ESR, erythrocyte sedimentation rate; IL, interleukin; Th17, T helper 17 cells; BAFF, B-cell activating factor; NA, not available; TBUT, tear film break-up time; OSDI, ocular surface disease index.
*Repeated-measures ANOVA; †Linear mixed model.

Changes in parameters

Changes in parameters are shown in Table 2 and Figs. 2 and 3. Regarding systemic inflammatory parameters, the mean ESR tended to continuously increase in the placebo group and decrease in the HCQ group, although there were no significant differences between the groups. The mean serum IL-6, BAFF, proportion of Th17 cells, and tear BAFF were not significantly different between the groups. Tear IL-17 was not detected in almost of all the samples.

With respect to the ocular signs and symptoms, TBUT did not change significantly from the baseline to week 12 in either group. The Schirmer test score did not significantly change in either group. The HCQ group tended to show continuous reduction of the corneal staining score, although the reduction was not significant. OSDI was significantly decreased between the baseline and weeks 6 and 12 only in the HCQ group ($P < 0.05$, baseline vs. 6 weeks; $P < 0.01$, baseline vs. 12 weeks; paired *t*-test; Fig. 3H), suggesting improvement of subjective symptoms. However, the HCQ and placebo groups were not significantly different ($P = 0.209$, repeated-measures ANOVA).

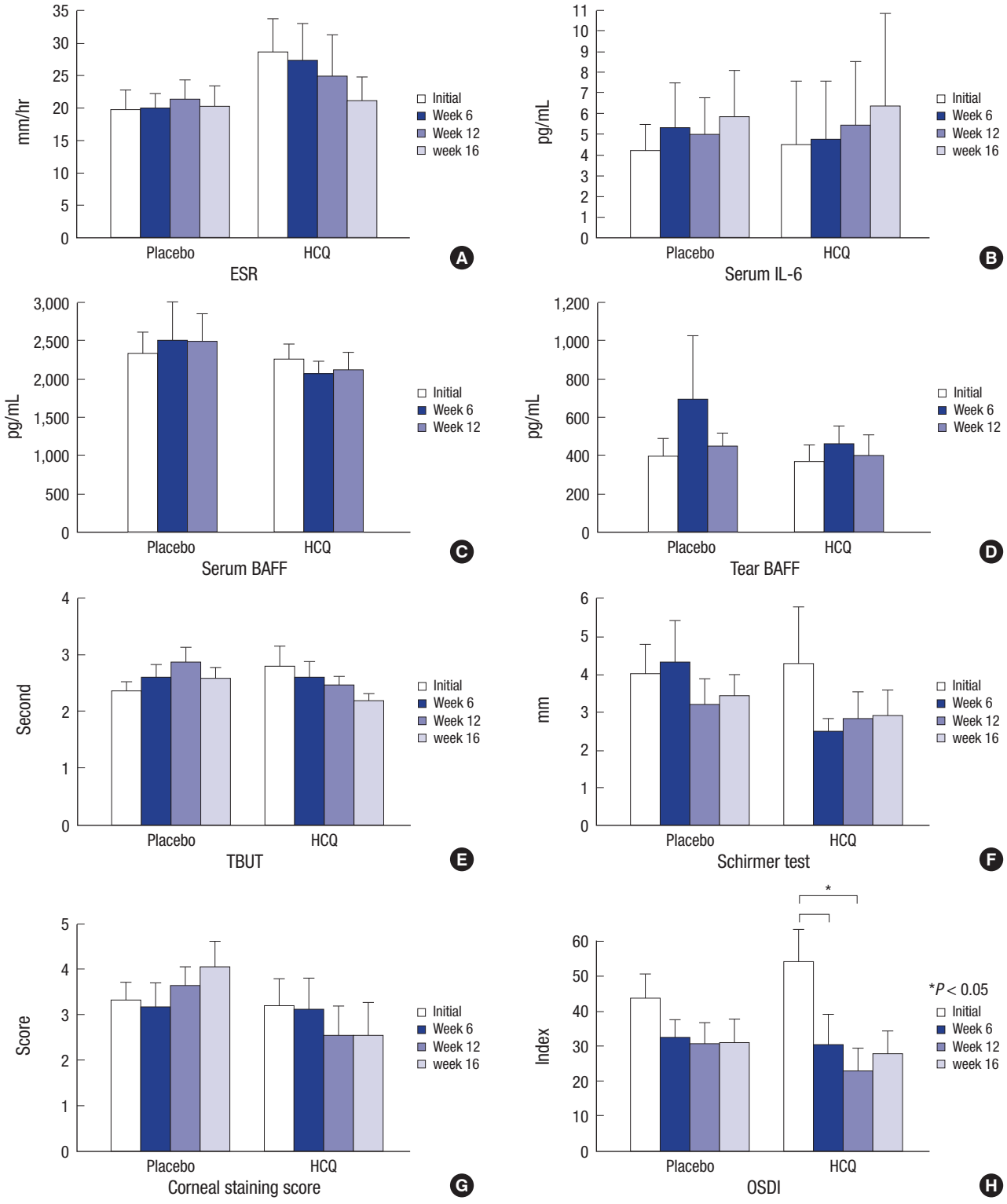

Fig. 3. Changes of ocular and systemic parameters in the hydroxychloroquine (HCQ) and placebo groups during follow-up. ESR (**A**) and serum IL-6 (**B**) levels, serum (**C**) and tear (**D**) BAFF levels, tear break-up time (TBUT) (**E**), Shirmer test score (**F**), corneal staining score (**G**), and ocular surface disease index questionnaire (OSDI) (**H**) are shown. Bars represent the mean + standard error.

After discontinuation of the oral medication, only the proportion of Th17 cells was significantly increased in both groups ($P < 0.01$, repeated-measures ANOVA). However, there was no difference between the groups ($P = 0.566$). Other parameters did not show significant changes after the oral medications were discontinued (Table 2, Fig. 2).

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DISCUSSION

No definite beneficial effect of the use of HCQ in the treatment of dry eye in pSS was found in this study, although there was evidence of improved subjective ocular symptoms. The study did not support an effect of HCQ treatment on tear production and inflammatory parameters such as ESR, IL-6, BAFF, and Th17 cell levels in pSS patients.

The main anti-inflammatory mechanism of HCQ is considered to be non-specific antagonism at TLR9 and TLR7 (16,17). Circulating DNA- and RNA-containing immune complexes in the blood may stimulate plasmacytoid dendritic cells (pDCs) through TLR9 and TLR7 (17,18). Activated pDCs produce IL-6, which can induce co-stimulatory molecules, and the stimulated co-stimulatory molecules combine with T cell receptor activate T helper (Th) cells (19,20). A recent study revealed that the number of Th17 cells is increased and that these cells are involved in the main pathogenic pathways in RA, SS, SLE, and GVHD (21,22), and many studies have shown that the IL-17 level is increased in the serum, saliva, and tears in SS patients (15,22-24). In addition, activated pDCs interact with B cells, which produce BAFF. BAFF is an essential homeostatic cytokine for B cells that regulates both innate and adaptive immunity (25). IL-6 and BAFF are over-expressed in SS patients (26). The lack of tear production in SS subjects limited our selection of inflammatory parameters. Therefore, we analyzed the levels of ESR, IL-6, Th17 cells and BAFF in the serum and those of BAFF and IL17 in tears.

We evaluated the effect of HCQ on dry eye and systemic changes of inflammatory parameters. Serum and tear BAFF levels were not significantly changed. In two previous reports on BAFF levels after HCQ treatment, serum BAFF levels were decreased after HCQ medication, and tear BAFF levels were increased after HCQ discontinuation (8,27). However, the patients of these two reports constantly took oral HCQ mediation for more than 2 years before enrollment. This could have introduced a bias in that only patients whose condition had improved after HCQ medication remained on HCQ medication at the time of enrollment. ESR and serum IL-6 levels were not significantly changed, which was comparable with a previous study (9). However, there are a few reports that ESR and serum IL-6 levels were significantly decreased after HCQ treatment (7,10). Possible reasons for the lack of change in ESR and IL-6 are as follows: 1) HCQ may have no clinically beneficial anti-inflammatory effect. 2) Short-term usage (12 weeks) may have been inadequate to stop the chronic inflammatory process and vicious cycle, although an effective drug concentration is reached in the serum within 2 weeks. 3) Previous studies of HCQ in SS patients permitted low doses of oral steroid or topical cyclosporine A (8,28). Our patients were not allowed any other oral or topical anti-inflammatory medication, which may have caused the results in our study to differ from those of the other studies. 4) The small number of enrolled subjects might have prevented the results from reaching statistical significance. HCQ showed no definite effect on Th17 cells, and the possible reasons are as mentioned above. In addition, the dosage of 300 mg daily may not be sufficient to ameliorate inflammation such as that involving activated Th17 cells.

Regarding ocular changes, the Schirmer test score did not change significantly during follow-up after HCQ treatment and showed no difference between the groups, which suggests that the HCQ treatment did not affect tear production in this study. The presumed reasons for this finding are as follows: 1) HCQ actually has no clinically beneficial anti-inflammatory effect. 2) Considering the age of the subjects (mean age, 56.8 years), most of the lacrimal gland may have been destroyed by chronic inflammation before treatment. HCQ treatment may thus only affect tear production in younger individuals with early inflammation.

The subjective symptoms represented by the OSDI score improved while taking the medication in the HCQ group, but the difference was insignificant between the groups. The small group size may explain the lack of significant difference between the two groups.

Taken together, the present findings show that HCQ medication did not significantly improve pSS during the study period. However, we did not investigate all of the anti-inflammatory cytokines and inflammatory cells because of the small quantities of collected tears and blood. Therefore, we may have missed some other anti-inflammatory function of HCQ.

There were several limitations in this study. First, the appropriate sample size to obtain statistical significance could not be achieved. Many of the pSS patients were already taking oral medications such as HCQ, pilocarpine, steroids, or other immunosuppressants and were reluctant to stop these medications for a sufficient wash-out period. Furthermore, budget constraints prevented prolonging the study to achieve the targeted sample size. Second, the study schedule was short. Previous studies that reported significant positive results of HCQ in pSS ran for 12 months (7,9,10) or included cessation of 3 months after 48 months or more of treatment (8). Third, the small number of enrolled patients may have prevented significant differences from being obtained. Fourth, symptoms (OSDI) and signs (Schirmer test) of patients in this study were milder than previous studies (8,29). Selection bias can affect the results. Nevertheless, our study is important because it supports other reports and provides evidence that HCQ does not have a definite beneficial effect on dry eye or systemic inflammation.

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DISCLOSURE

The authors have no potential conflicts of interest to disclose.

AUTHOR CONTRIBUTION

Research conception & design: Yoon CH, Lee HJ, Lee EY, Lee EB, Lee WW, Kim MK, Wee WR. Performing the experiments: Yoon CH, Lee HJ, Lee WW, Kim MK. Data acquisition: Lee EY, Lee EB, Kim MK, Lee WW. Data analysis and interpretation: Yoon CH, Lee HJ, Kim MK, Lee WW. Statistical analysis: Yoon CH, Kim MK. Drafting of the manuscript: Yoon CH, Kim MK. Critical revision of the manuscript: Yoon CH, Kim MK. Receiving grant: Kim MK. Approval of final manuscript: all authors.

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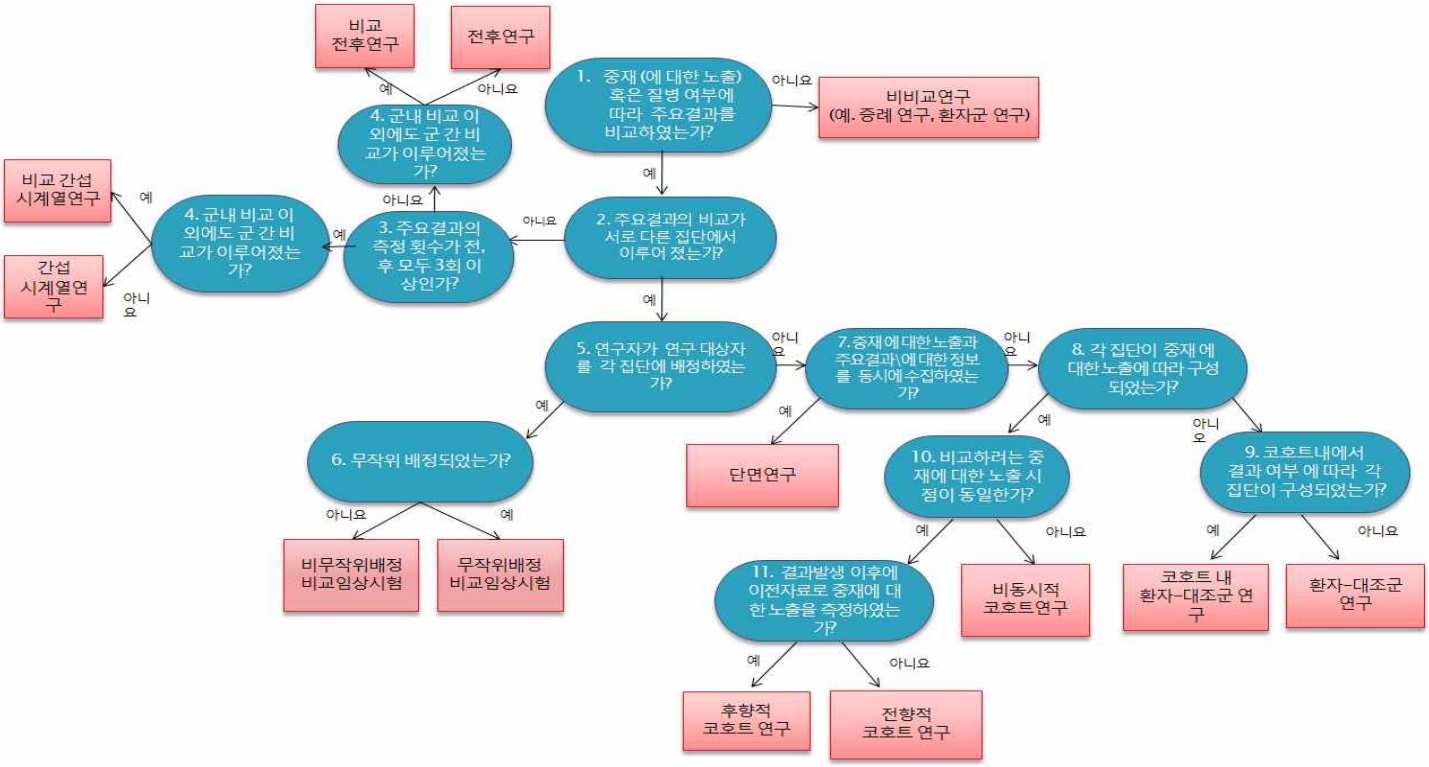
[실습자료3]

DAMI 2.0



ROBANS 도구

영역	설명	비뚤임 위험	판단 근거(논문에서 그대로 인용함)
대상군 비교가능성	비교가 부적절한 대상군 선정으로 인해 발생한 선택 비뚤임	□ 낮음 □ 높음 □ 불확실	
대상군 선정	부적절한 중재 혹은 노출군 또는 환자군 선정으로 발생한 선택 비뚤임	□ 낮음 □ 높음 □ 불확실	
교란변수	교란변수 확인과 고려가 부적절하여 발생한 선택 비뚤임	□ 낮음 □ 높음 □ 불확실	
노출 측정	부적절한 중재 혹은 노출 측정으로 인해 발생한 실행 비뚤임	□ 낮음 □ 높음 □ 불확실	
평가자의 눈가림	부적절한 평가자 눈가림으로 인해 발생한 확인 비뚤임	□ 낮음 □ 높음 □ 불확실	
결과 평가	부적절한 결과 평가 방법로 인해 발생한 결과 확인 비뚤임	□ 낮음 □ 높음 □ 불확실	
불완전한 결과자료	불완전한 자료를 부적절하게 다루어 발생한 탈락 비뚤임	□ 낮음 □ 높음 □ 불확실	
선택적 결과 보고	선택적 결과 보고 때문에 발생한 보고 비뚤임	□ 낮음 □ 높음 □ 불확실	

1. 대상군 비교가능성 비교가 부적절한 대상군 선정으로 인해 발생한 선택 비뚤임	
비뚤임 위험 '낮음' 기준	**코호트 연구** 중재에 대한 노출군과 비교군이 적응증과 질병의 중증도 등에 차이가 없어 비교할 만한 인구집단이다. **단면연구** 비교하려는 두 군이 적응증과 질병중등도 등에 차이가 없어 비교할 만한 인구집단이다. **환자-대조군 연구** 환자군과 대조군이 중재에 대한 노출의 가능성에 차이가 없어 비교할 만한 인구집단이다. **전후연구** 중재에 대한 노출 전후의 인구집단이 동일하다.

비뚤임 위험 ‘높음’ 기준	**코호트 연구** 중재에 대한 노출군과 비교군이 질병의 중증도, 적응증 등에서 차이가 있어 비교할만한 인구집단이 아니다. **단면연구** 비교하려는 군간 질병중등도나 적응증에 차이가 있어 비교할 만한 인구집단이 아니다. **환자-대조군 연구** 환자군과 대조군이 중재에 대한 노출 가능성에 차이가 있어 비교할 만한 인구집단이 아니다. **전후연구** 중재에 대한 노출 전후의 인구집단이 동일하지 않다.
비뚤임 위험 ‘불확실’ 기준	대상군 비교가능성에 대한 비뚤임 위험이 ‘낮음’, ‘높음’ 중 어디에 해당하는지 불확실한 경우

2. 대상군 선정 부적절한 중재 혹은 노출군 또는 환자군 선정으로 발생한 선택 비뚤임	
비뚤임 위험 ‘낮음’ 기준	해당 연구 설계 별로 대상군 선정에 아래 두 가지 조건을 모두 만족하는 경우 **코호트 연구** 연구 참여 시점에 연구 대상자에서 결과가 없음을 확인하였다. 참여자 모집전략(포함/배제 기준, 선정 방법)이 대상군 모두에서 동일하다. **단면연구** 연구 참여 시점에 결과 발생여부가 대상자 선정에 영향을 미치지 않았음을 확인하였다. 참여자 모집전략(포함/배제 기준, 선정 방법)이 대상군 모두에서 동일하다. **환자-대조군 연구** 대조군은 질병이 없다는 사실을 확인하였다. 일반인구 집단에서 표본을 추출하였다. **전후 연구** 대상군은 연속적(consecutive)으로 모집하였다. 자료를 전향적으로 수집하였다.
비뚤임 위험 ‘높음’ 기준	다음 중 한 가지 이상에 해당되는 경우 **코호트 연구** 연구 참여 시점에 연구 대상자에서 결과가 없음을 확인하지 못하였다. 참여자 모집전략(포함/배제 기준, 선정 방법)이 대상군에 따라 다르다. **단면연구** 연구 참여 시점에 연구 대상자에서 발생한 결과가 대상군 선정에 영향을 주지 않았는지 확인하지 못하였다. 참여자 모집전략(포함/배제 기준, 선정 방법)이 대상군에 따라 다르다. **환자-대조군 연구** 일반인구집단에서 표본을 추출하지 못하였다. 대조군은 질병이 없음을 확인하지 못하였다. **전후 연구** 대상군을 연속적으로 모집하지 않았다. 후향적으로 자료를 수집하였다.

비뚤임 위험 '불확실' 기준	대상군 선정에 대한 비뚤임 위험이 '낮음', '높음' 중 어디에 해당하는지 불확실한 경우

3. 교란변수 교란변수 확인과 고려가 부적절하여 발생한 선택 비뚤임	
비뚤임 위험 '낮음' 기준	다음 중 한 가지 이상에 해당되는 경우 **전후 연구이외의 비무작위 연구** 주요 교란변수를 확인하였고 이를 설계 단계(짝짓기, 참여제한 등)에서 적절히 고려하였다. 주요 교란변수를 확인하였고 이를 분석 단계(층화, 성향점수(propensity score), 통계적 보정(회귀분석 등))에서 적절하게 보정하였다. **전후 연구** 질병, 중재 등의 특성상 시간경과에 다른 전후 차이(자연 경과)를 배제할 수 있다. 배제할 수 없으나 분석단계(회귀분석 등)에서 보정하였다.
비뚤임 위험 '높음' 기준	다음 중 한 가지 이상에 해당되는 경우 전후 연구이외의 비무작위 연구 주요 교란변수를 설계 단계나 분석단계에서 다루지 않았다. 주요 교란변수를 확인하였지만 이를 디자인 단계나 분석 단계에서 적절히 고려하지 못하였다. **전후 연구** 질병, 중재 등의 특성을 고려할 때 시간경과에 따른 전후 차이(자연 경과) 가 주요 결과에 영향을 줄 수 있음을 배제할 수 없고, 분석단계에서 고려하지 않았다.
비뚤임 위험 '불확실' 기준	교란변수에 대한 비뚤임 위험이 '낮음', '높음' 중 어디에 해당하는지 불확실한 경우

4. 노출 측정 부적절한 노출 측정으로 인해 발생한 실행 비뚤임	
비뚤임 위험 '낮음' 기준	노출 측정을 다음 두 가지를 모두 만족하는 경우 의무기록, 구조화된 인터뷰 등 신뢰할 수 있는 출처에서 확인하였다. 2회 이상 측정, 다수의 연구자가 독립적으로 측정, 노출 측정의 표준화 등 측정의 객관화, 표준화를 적절하게 하였다
비뚤임 위험 '높음' 기준	다음 중 한 가지 이상에 해당되는 경우 단순한 자가 응답으로 노출을 측정하였다. 비구조화 인터뷰 등으로 노출을 측정하였다. 회상 비뚤임(recall bias)이 비교적 명백하다. 2회 이상 측정, 다수의 연구자가 독립적으로 측정, 노출 측정의 표준화 등 측정의 객관화, 표준화 노력을 하지 않았거나 적절하지 않다.
비뚤임 위험 '불확실' 기준	노출 측정에 대한 비뚤임 위험이 '낮음', '높음' 중 어디에 해당하는지 불확실한 경우

5. 평가자의 눈가림 부적절한 평가자 눈가림으로 인해 발생한 확인 비뚤임	
비뚤임 위험 ‘낮음’ 기준	다음 중 한 가지 이상에 해당되는 경우 **환자-대조군 연구 이외의 비무작위 연구** 결과평가자의 눈가림이 적절하게 이루어졌으며 눈가림이 깨지지 않을 것이라 판단된다. 결과평가자 눈가림은 없지만 눈가림 여부가 결과 측정에 영향을 미치지 않는 것으로 판단된다. **환자-대조군 연구** 노출 평가자의 눈가림이 적절하게 이루어졌으며 눈가림이 깨지지 않을 것이라 판단된다. 노출 평가자 눈가림은 없지만 눈가림 여부가 노출 측정에 영향을 미치지 않는 것으로 판단된다.
비뚤임 위험 ‘높음’ 기준	다음 중 한 가지 이상에 해당되는 경우 **환자-대조군 연구 이외의 비무작위 연구** 결과평가자의 눈가림이 이루어지지 않았다. 결과 평가자의 눈가림이 이루어졌지만, 눈가림이 깨졌을 개연성이 있으면서 눈가림 여부가 결과 측정에 영향을 미칠 것으로 판단된다. **환자-대조군 연구** 노출 평가자의 눈가림이 이루어지지 않았다. 노출 평가자의 눈가림이 이루어졌지만, 눈가림이 깨졌을 개연성이 있으면서 눈가림 여부가 노출 측정에 영향을 미칠 것으로 판단된다.
비뚤임 위험 ‘불확실’ 기준	평가자의 눈가림에 대한 비뚤임 위험이 ‘낮음’, ‘높음’ 중 어디에 해당하는지 불확실한 경우

6. 결과 평가 부적절한 결과 평가 방법으로 인해 발생한 결과 확인 비뚤임	
비뚤임 위험 ‘낮음’ 기준	다음 중 한 가지 이상에 해당되는 경우 신뢰도와 타당도가 입증된 도구 등을 사용하여 환자 보고 결과(patient reported outcome)를 평가하였다. 검사결과(results)나 혈압 등 측정을 통해 얻어지는 결과인 경우 측정 장비의 정확성 인증을 시행하였다. 사망이나 질환 발생 등의 결과인 경우 의무기록이나 신뢰할 수 있는 자료원에서 확인하였다. 기타 결과에서 타당도가 검증된 도구나 객관적인 평가방법으로 사용하여 결과 평가가 신뢰성 있는 방법으로 이루어졌다고 판단된다.
비뚤임 위험 ‘높음’ 기준	다음 중 한 가지 이상에 해당되는 경우 단순한 자가보고만으로 환자 보고 결과(patient reported outcome)을 평가하였다. 검사결과(results)나 혈압 등 측정을 통해 얻어지는 결과인 경우 측정 장비의 정확성 인증이 시행되지 않았다. 사망이나 질환 발생 등의 결과인 경우 의무기록이나 신뢰할 수 있는 자료원을 확인하지 않았다. 기타 결과에서 타당도가 검증된 도구나 객관적인 평가방법으로 사용하지 않아 결과 평가가 신뢰성 있는 방법으로 이루어지지 않았다.
비뚤임 위험 ‘불확실’ 기준	결과 평가에 대한 비뚤임 위험이 ‘낮음’, ‘높음’ 중 어디에 해당하는지 불확실한 경우

7. 불완전한 자료 불완전한 자료를 부적절하게 다루어 발생한 탈락 비뚤임	
비뚤임 위험 ‘낮음’ 기준	다음 중 한 가지 이상에 해당되는 경우 **전후 연구이외의 비무작위 연구** 결측치가 없다. 결측치가 생긴 이유가 결과에 주는 영향이 없을 것이라 판단된다. 결측치가 중재 혹은 노출군-대조군간 유사하게 발생하고 결측치가 생긴 이유도 유사하다. **전후 연구** 탈락자와 완료자의 기저상태가 차이가 없다.
비뚤임 위험 ‘높음’ 기준	다음 중 한 가지 이상에 해당되는 경우 **전후 연구이외의 비무작위 연구** 두 군에서 불완전자료의 비율 혹은 이유가 차이가 나면서, 결측치가 생긴 이유 때문에 결과에 다른 영향을 미칠 것이라 판단된다. **전후 연구** 탈락자와 완료자의 기저상태가 차이가 있다.
비뚤임 위험 ‘불확실’ 기준	불완전한 자료에 대한 비뚤임 위험이 ‘낮음’, ‘높음’ 중 어디에 해당하는지 불확실한 경우

8. 선택적 결과 보고 선택적 결과 보고 때문에 발생한 보고 비뚤임	
비뚤임 위험 ‘낮음’ 기준	다음 중 한 가지 이상에 해당되는 경우 프로토콜이 존재하고 사전에 정의한 일차, 이차 결과가 계획했던 대로 기술되어 있다. 프로토콜은 없지만 예상되는 거의 모든 주요 결과를 포함하고 있다.
비뚤임 위험 ‘높음’ 기준	다음 중 한 가지 이상에 해당되는 경우 사전에 정의한 일차, 이차 결과 중 보고되지 않은 것이 있다. 정해지지 않은 방법으로 결과 보고가 이루어졌다. 사전에 정의되지 않은 결과가 보고되었다 (보고 하는 명백한 이유 설명이, 있는 경우는 예외). 해당 분야 연구에서 보고될 것으로 예상되는 주요 결과에 대한 보고가 없다.
비뚤임 위험 ‘불확실’ 기준	선택적 결과 보고에 대한 비뚤임 위험이 ‘낮음’, ‘높음’ 중 어디에 해당하는지 불확실한 경우

Efficacy of Influenza Vaccine in Nursing Homes

Reduction in Illness and Complications During an Influenza A (H3N2) Epidemic

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• **From December 10, 1982, to March 4, 1983, when influenza A (H3N2) viruses circulated in Michigan, outbreaks of influenza-like illness were identified in seven nursing homes in Genesee County; 272 (27%) of 1,018 residents were affected. Unvaccinated residents were more likely than vaccinated residents to become ill (risk ratio [RR], 2.6; 95% confidence interval [CI], 1.8-3.6) and were subsequently more likely to be hospitalized (RR, 2.4; 95% CI, 1.2-4.8), develop roentgenographically proven pneumonia (RR, 2.9; 95% CI, 1.6-5.3), or die (RR, 5.6; 95% CI, 1.2-9.1). Similar observations were made during investigations in six of the eight remaining nursing homes in Genesee County, in which 57 (12%) of 458 residents became ill sporadically. These findings suggest that influenza vaccine can reduce the incidence and severity of influenza virus infections among the elderly and chronically ill and underscore the importance of vaccination programs for those in nursing homes and in the general community.**

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STUDIES of the general population and analyses of mortality data have consistently shown that elderly persons and those with certain chronic medical conditions are at greatest risk of death or other serious complications following influenza virus infections.[1-4] As a logical consequence of these observations, the Immunization Practices Advisory Committee[5] and others[6] have strongly recommended that such persons be immunized against influenza annually, based on extrapolation of efficacy data for younger individuals.[7] There is, however, very little direct evidence to substantiate the effectiveness of immunization for high-risk groups.[8,9]

During the winter of 1982-1983, influenza viruses related to A/Bangkok/1/79 (H3N2) circulated in Michigan and caused widespread outbreaks in its lower peninsula.[10] Because Michigan law requires that nursing homes document all cases of acute respiratory illness, we were able to study systematically the impact of the epidemic on a large, well-defined population of elderly people and to estimate retrospectively the efficacy of vaccination (A/Bangkok/1/79 antigen) in reducing morbidity and mortality.

BACKGROUND AND METHODS

Active surveillance of influenza in Michigan is routinely conducted from November through April and includes weekly reports and laboratory specimens collected from schools, acute-care hospitals, and sentinel physicians. Based on reports of widespread influenza A (H3N2) activity in the community from December 1982 to March 1983 and reports of outbreaks in seven of 15 nursing homes, Genesee County (Flint metropolitan area) was selected for further study. All nursing homes in the county had conducted influenza vaccination programs during the previous fall, when each attending physician ordered the currently recommended, commercially available vaccine (containing 15 μg each of hemagglutinin of A/Bangkok/79 [H3N2], A/Brazil/78 [H1N1], and B/Singapore/79) for all residents under his or her care. The only residents who were not vaccinated were those who refused or whose relatives had not given consent (required by all 15 nursing homes).

During April and May 1983, data concerning each resident with influenza-like illness were recorded on standardized forms by infection-control nurses at each of 14 homes that agreed to participate in the study. The number of residents who had received influenza vaccine the previous fall, as well as the number who required skilled or intermediate nursing care, were also provided for each home. During the study period, the average monthly census of each home had not varied by more than 5%, with mortality rates ranging from 0% to 6.2%.

In May and June, we reviewed medical records at these homes to verify the information recorded for each resident with influenza-like illness (98% of charts were available for review) and to ascertain additional cases that had not been reported (by reviewing a 10% random sample of the remaining charts). As a result of this review, one home (71 residents) was excluded from the study; the data could not be confirmed independently since fever and other signs and symptoms of acute illness had not been routinely documented in the medical records.

For purposes of uniform data collection, subsequent analyses, and presentation of findings in this report, we used the following definitions. "Influenza-like illness" was defined by a temperature of 37.8 °C or greater, accompanied by cough, coryza, or sore throat. An "outbreak" was defined by an overall attack rate of at least 10% in any nursing home within any seven-day period from December 10, 1982, to March 4, 1983 (the interval when influenza viruses related to A/Bangkok/1/79 [H3N2] were known to be circulating in the community; Figure). The "duration" of an outbreak was defined by the number of days in which one or more cases of influenza-like illness occurred, with no more than two consecutive days when there were no additional cases. All residents who had onset of illness during an out-

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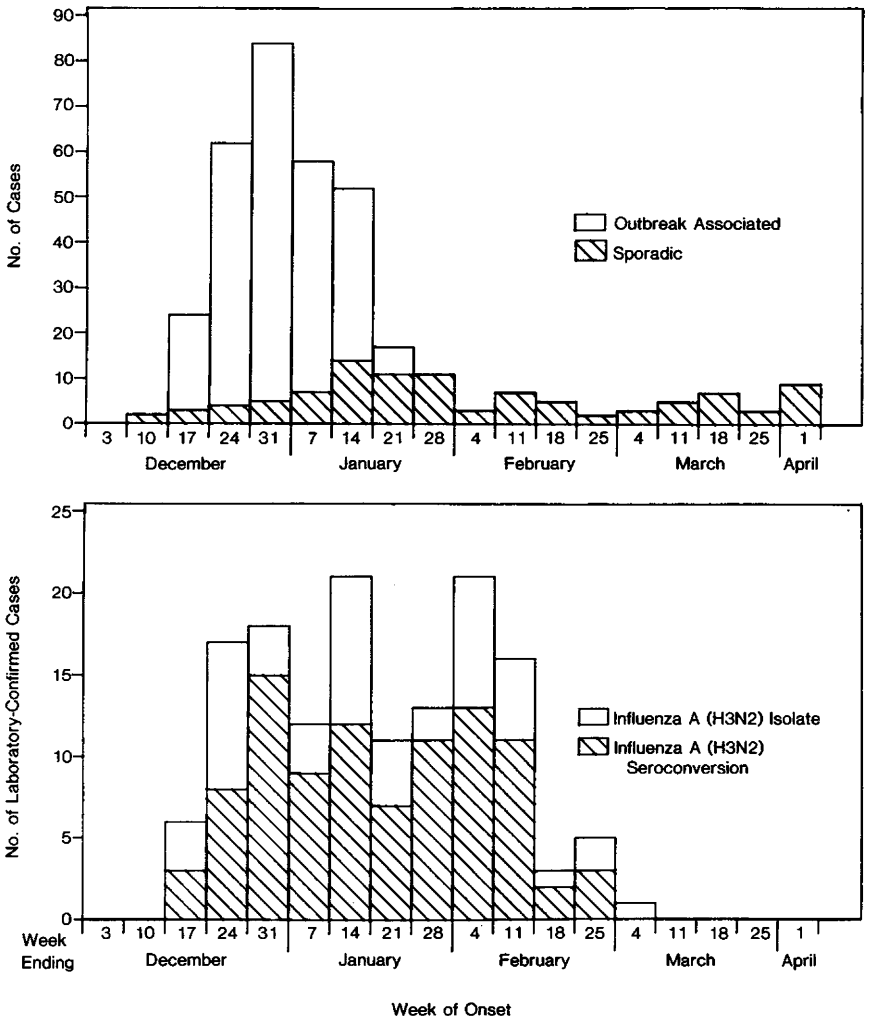

Cases of influenza-like illness in nursing homes (Genesee County) (top) and laboratory-proven influenza infections in community (all counties) (bottom) by week of onset, Michigan, November 27, 1982, to April 1, 1983.

break were classified as "outbreak-associated cases," while the remainder were classified as "sporadic." Illness was accompanied by "pneumonia" when one or more areas of acute interstitial or alveolar infiltrates were documented roentgenographically. "Influenza-associated deaths" were those that occurred within two weeks of onset of influenza-like illness, with no intervening asymptomatic period and no apparent alternative explanation.

Reductions in incidence of illness, hospitalization, pneumonia, and death that were ascribed to influenza vaccination assume that vaccinated and unvaccinated groups were otherwise comparable. Since the validity of vaccine efficacy calculations[8] also depends on the assumption that all residents were at equal risk of exposure to influenza viruses throughout the study period, we analyzed the 1,018 residents in the seven homes with outbreaks separately from the 458 residents in the six homes without outbreaks (see below). Finally, in an effort to account for other variables that differed from home to home (eg, the total number of residents), we considered each nursing home as a separate stratum and used the Mantel-Haenszel χ^2 test[11] for analysis, unless noted otherwise.

RESULTS

Outbreak-Associated and Sporadic Cases of Influenza-like Illness

In the period December 10, 1982, to March 4, 1983, we identified 329 cases of influenza-like illness among 1,476 residents of 13 nursing homes included in the study (Fig 1). Of the 329 cases, 251 (76%) were associated with outbreaks in seven of these homes and 78 (24%) occurred sporadically—21 within the seven homes that had outbreaks and 57 within the six homes that did not (Table 1). None of the 329 residents had influenza-like illness more than once during the study period.

Epidemiologic features of illness were similar in all 13 homes. All seven outbreaks occurred from mid-December through late January—coincident with the peak of febrile respiratory illness in the community (not shown)—and lasted from 12 to 23 days (median, 18 days). Sporadic illnesses often clustered within seven-day periods (not shown), with the majority of cases occurring in December (18 cases) and January (42 cases). As shown in Table 1, the attack rate of influenza-like illness was similar for residents who required skilled care *v* those who required intermediate care. Overall age-specific attack rates could not be determined, since age had been recorded only for cases.

Four of five nasopharyngeal and throat swabs collected during three of the outbreaks (homes A, B, and D) yielded influenza viruses similar to A/Bangkok/1/79 (H3N2) (a strain included in the vaccine); six of eight other residents in these homes had fourfold or greater rises in hemagglutination-inhibition antibody against influenza A (H3N2) viruses but no comparable rises against other respiratory pathogens. Specimens for laboratory confirmation were not available from the four remaining outbreaks nor from residents with sporadic illness. Although there were laboratory-proven influenza A infections in both nursing homes and in the general community, none of the homes in the study had used amantadine hydrochloride for prophylactic or therapeutic purposes.

Reduction of Influenza-like Illnesses and Associated Complications During the Study Period That Were Attributable to Influenza Vaccination

Reduction of Illness.—As shown in Table 2, unvaccinated residents had higher attack rates than vaccinated residents in six of the seven homes with outbreaks and were more than twice as likely to develop influenza-like illness overall ($P<.0001$, Mantel-Haenszel χ^2 test [$MH\chi^2$]). Overall attack rates were also higher for unvaccinated residents in the six homes with no outbreaks (17% of 119 *v* 11% of 339 vaccinated residents; $P<.02$, $MH\chi^2$). These differences could not be explained on the basis of disparities in age (mean ± SD, 82.3 years ± 9.3 *v* 80.4 years ± 11.8) or the proportion requiring skilled nursing care (27.6% *v* 31.5%) in the vacci-

Table 1.—Outbreak-Associated and Sporadic Cases of Influenza-like Illness by Level of Required Nursing Care, Genesee County (Michigan) Nursing Homes, December 10, 1982, to March 4, 1983

Nursing Home	No. of Residents	No. (%) of Outbreak-Associated Cases	No. (%) of Sporadic Cases	Skilled Care*			Intermediate Care*		
				No. of Residents	No. of Cases	% Ill	No. of Residents	No. of Cases	% Ill
A†	120	26 (21.7)	4 (3.3)	52	11	21.2	68	19	27.9
B†	164	45 (27.4)	2 (1.2)	115	22	19.1	49	25	51.0
C	211	37 (17.5)	6 (2.4)	33	7	21.2	178	36	20.2
D†	148	21 (14.2)	1 (0.7)	1	0	0.0	147	22	15.0
E	180	43 (23.9)	5 (2.8)	85	37	43.5	95	11	11.6
F	94	28 (29.8)	2 (2.1)	21	8	38.1	73	22	30.1
G	101	51 (50.5)	1 (1.0)	0	...	...	101	52	51.5
H	107	...	7 (6.5)	5	0	0.0	102	7	6.9
I	92	...	10 (10.9)	32	5	15.6	60	5	8.3
J	91	...	16 (17.6)	30	4	13.3	61	12	19.7
K	96	...	13 (13.5)	15	3	20.0	81	10	12.3
L	25	...	2 (8.0)	0	...	...	25	2	8.0
M	47	...	9 (19.1)	0	...	...	47	9	19.1
Total	1,476	251 (17.0)	78 (5.3)	389	97	24.9	1,087	232	21.3

*Determined by nursing home administrators, based on standard Medicare and Medicaid criteria.[12]
†Laboratory-confirmed outbreaks of influenza viruses related to A/Bangkok/1/79 (H3N2).

Table 2.—Cases of Influenza-like Illness (ILI) and Associated Complications in Seven Genesee County (Michigan) Nursing Homes With Outbreaks, December 10, 1982, to March 4, 1983*

Nursing Home	No. (%) of Residents		No. (%) of Residents With ILI		No. (%) of Residents Hospitalized for ILI		No. (%) of Residents Who Developed Pneumonia Following ILI		No. (%) of Residents Who Died Following ILI	
	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated
A	74 (62)	46 (38)	11 (15)	19 (41)	0 (0)	1 (2.2)	1 (1.4)	8 (17.4)	0 (0)	6 (13.0)
B	144 (88)	20 (12)	40 (28)	7 (35)	4 (2.8)	1 (5.0)	3 (2.1)	3 (15.0)	2 (1.4)	0 (0)
C†	85 (40)	126 (60)	8 (9)	31 (25)	2 (2.4)	6 (4.8)	1 (1.2)	6 (4.8)	0 (0)	1 (0.8)
D	40 (27)	108 (73)	8 (20)	14 (13)	0 (0)	1 (0.9)	0 (0)	1 (0.9)	0 (0)	0 (0)
E	120 (67)	60 (33)	15 (13)	33 (55)	4 (3.3)	13 (21.7)	5 (4.2)	12 (20.0)	1 (0.8)	7 (11.7)
F	33 (35)	61 (65)	7 (21)	23 (38)	1 (3.0)	7 (11.5)	2 (6.1)	9 (14.8)	1 (3.0)	6 (9.8)
G	52 (51)	49 (49)	24 (46)	28 (57)	8 (15.4)	2 (4.1)	10 (19.2)	6 (12.2)	2 (3.8)	1 (2.0)
Total	548 (54)	470 (46)	113 (21)	155 (33)‡	19 (3.5)	31 (6.6)§	22 (4.0)	45 (9.6)‖	6 (1.1)	21 (4.5)¶

*Results changed only negligibly when the 20 sporadic cases in these homes were excluded from analysis.
†Four cases excluded since vaccination status could not be determined.
‡Significantly higher *v* vaccinated group; $P<.0001$ (Mantel-Haenszel χ^2 test); risk ratio (RR), 2.6 (95% confidence interval [CI], 1.8-3.6).
§Significantly higher *v* vaccinated group; $P<.01$ (Mantel-Haenszel χ^2 test); RR, 2.4 (95% CI, 1.2-4.8).
‖Significantly higher *v* vaccinated group; $P<.001$ (Mantel-Haenszel χ^2 test); RR, 2.9 (95% CI, 1.6-5.3).
¶Significantly higher *v* vaccinated group; $P<.001$ (Mantel-Haenszel χ^2 test); RR, 5.6 (95% CI, 1.2-9.1).

nated group *v* the unvaccinated group, respectively.

Reduction of Complications.—As shown in Table 2, unvaccinated residents were more likely than vaccinated residents to be hospitalized ($P<.01$) and/or develop pneumonia ($P<.001$) in the seven homes with outbreaks. Similar trends were observed in the remaining six homes, but none were statistically significant. Unvaccinated residents were also more likely to die of influenza-like illness, not only in the homes with outbreaks (Table 2) but also in the six homes with no outbreaks (3.4% of 119 *v* 0.6% of 339 vaccinated residents; $P<.05$, MHχ^2). On the basis of these data, influenza vaccination was associated with a 79% reduction in mortality overall, similar to the estimate for elderly persons living in the community.[8]

Influenza vaccine also appeared to reduce the risk of pneumonia and death even when it failed to prevent influenza-like illness: whereas 26 (17%) of 150 vaccinated cases developed pneumonia and eight (5%) died, 47 (27%) of 175 unvaccinated cases developed pneumonia and 25 (14%) died ($P<.05$ for each comparison, χ^2 test). These findings could not be explained on the basis of differences in age, debility (as measured by level of nursing care), the type or duration of antibiotic therapy, rates of hospitalization, or pneumococcal vaccination status between vaccinated and unvaccinated cases.

COMMENT

This investigation documents the often devastating consequences of influenza infections in nursing homes and evaluates the effectiveness of influenza vaccine in reducing the incidence of illness and associated complications. As was found in the collective experience of previous studies,[7,9] the efficacy of the vaccine in preventing illness varied widely from home to home, for reasons that remain unknown. The overall efficacy in the present study (approximately 37% in homes with outbreaks and 28% in others) was also comparable with overall estimates based on other investigations of influenza A (H3N2) outbreaks[9,13-17] but considerably lower

than estimates for younger age groups.[7] Thus, present knowledge seems to indicate that influenza vaccine may not provide optimal protection against infection for the elderly and chronically ill. Nevertheless, the results of the present study also suggest, more importantly, that vaccination may attenuate infection: compared with vaccinated persons, unvaccinated persons were more likely to be hospitalized, develop pneumonia, and/or die of influenza-like illness. These differences in outcome are unlikely to be due to chance alone and cannot be readily explained by other factors such as noncomparability of study groups, ascertainment biases, or a nonspecific case definition, for the following reasons:

First, available data suggest that the vaccinated and unvaccinated groups were otherwise comparable. Since influenza vaccine was offered to all residents in each nursing home on a voluntary basis (following authorization from a family member), there would have to have been a consistent bias to withhold the vaccine from older, more debilitated residents to explain the poor outcome of the unvaccinated group. The fact that vaccinated and unvaccinated cases were similar in age and required a comparable level of nursing care suggests that such biases did not exist, a conclusion supported by a separate study of 4,865 residents in 40 randomly or systematically selected nursing homes in the Midwest, including 1,582 residents in 14 nursing homes in Michigan (Centers for Disease Control, unpublished data, November 1982). Furthermore, significant differences in the incidence of pneumonia and death between unvaccinated and vaccinated residents who did acquire influenza-like illness could not be attributed to differences in age, level of nursing care, pneumococcal vaccination status, or therapy.

Second, independent record reviews suggest that there was no bias in preferentially ascertaining cases from the unvaccinated population. Although we were unable to identify precisely those residents who were present in each home throughout the study period, it is unlikely that the incidence of illness or complications in the unvaccinated group were artificially elevated, since there was relatively little turnover in the resident population.

Finally, although few infections were laboratory confirmed, indirect evidence suggests that the majority of illnesses were caused by influenza. The fact that each resident had no more than one febrile respiratory illness suggests that our case definition was relatively specific, particularly during the time when influenza viruses were known to be circulating widely in the community. Epidemiologic features of outbreak-associated and sporadic cases were similar and were also consistent with previous reports of laboratory-confirmed influenza A (H3N2) infections in nursing homes.[9,13-17] Parainfluenza and respiratory syncytial virus infections have occasionally been documented in this setting, but outbreaks are typically indolent and have rarely been associated with fatal cases.[17-20] Indeed, assuming that these or other agents did account for at least some of the cases in the present investigation, the efficacy of influenza vaccine may have been underestimated, since it would not influence the acquisition or outcome of infections caused by pathogens other than influenza.

In spite of numerous studies that have documented the severity of influenza infections in high-risk groups and the recommendations of the Immunization Practices Advisory Committee over the past two decades, relatively few of the estimated 48 million elderly and/or chronically ill persons in the United States—including 1.3 million residents in 18,900 nursing homes[12]—receive influenza vaccine in any given year.[21] The reasons for such a low rate of acceptance have not been fully defined but may be attributable in part to uncertainty about the effectiveness of the vaccine. The present study, however, provides some evidence to show that influenza vaccine can influence the acquisition and outcome of infections caused by closely related viruses and supports the view that the impact of influenza in the United States can be reduced if substantial numbers of high-risk individuals are vaccinated.

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