Evaluation of the effectiveness of colchicine therapy in preventing renal amyloidosis in patients with Familial Mediterranean Fever in Yerevan, Armenia

Master of Public Health Thesis Project Utilizing Professional Publication Framework

Maria Sevoyan, MD
MPH Candidate

College of Health Sciences
American University of Armenia

Primary Adviser: Haroutune Armenian, MD, Dr.PH
Secondary Adviser: Krikor Soghikian, MD, MPH

Yerevan, Armenia
October 2005

Table of Contents

Acknowledgments

I would like to express my deepest gratitude to my adviser Dr. Haroutune Armenian for his insightful comments and helpful suggestions during my thesis project preparation. I acknowledge my adviser Dr. Krikor Soghikian for his interest and help in designing the project.

I am also greatly thankful to Dr. Varduhi Petrosyan for her helpful comments and valuable advice.

I would like to express my deepest and sincere thanks to Gayane Yenokyan, her kind attitude and support was essential when I analyzed the study results and wrote the report.

I am also grateful to the administration of the Center of Medical Genetics, to Dr. Tamara Sarkissian, and to Ara Beglaryan and Gohar Shahsuvaryan for their knowledgeable recommendations and support.

Also thanks to Yelena Amirkhanyan for providing very important and valuable literature regarding familial Mediterranean fever.

I am thankful to all my friends and Public Health students, especially Gayane Ghavalyan who was very supportive to me.

I would like to thank my family members for their support and encouragement.

Abstract

Objectives

The study aimed to determine the association between colchicine therapy including the effect of duration and initiation of the treatment, dose and mode of colchicine use and risk of amyloidosis among patients with familial Mediterranean fever in Yerevan, Armenia.

Study Methods and Design

The study utilized case-control design. Thirty-three FMF patients with amyloidosis developed during the period 08/2003-08/2005 and 66 randomly selected FMF patients without amyloidosis were included in the study as cases and controls, respectively. The study participants were genetically verified patients with FMF selected from the Center of Medical Genetics' register. Both phone and face-to-face interviews were used for data collection.

Results

The analysis showed that the risk of amyloidosis decreased with adequate colchicine use versus non-adequate use (adjusted for gender and age: OR = 0.33; 95% CI 0.12-0.93); permanent colchicine use versus use with interruption (adjusted for gender and age: OR = 0.29; 95% CI 0.10-0.82), earlier versus later age at initiation of colchicine treatment (adjusted for FMF onset and gender: OR = 1.06; 95% CI 1.01-1.11); current colchicine users versus ever/never users (adjusted for gender and age: OR = 0.27; 95% CI 0.08-0.95). For current use and permanent use the protective effect was strengthening after controlling for age, gender, family history of amyloidosis and M694V mutation, OR = 0.20; 95% CI 0.05 - 0.83 and OR = 0.14; 95% CI 0.04 - 0.53, respectively.

Conclusion

The study demonstrated evidence that colchicine treatment is effective in preventing amyloidosis in Armenian FMF patients. Earlier start of initiation of colchicine treatment by FMF patients needs to be emphasized by the physicians. Regular and lifelong therapy with colchicine in adequate dose 1.2-1.8 mg per day has to be recommended to all FMF patients in order to prevent the FMF-associated amyloidosis development.

Introduction

Literature review

Description of the disease

Familial Mediterranean fever (FMF), also known as periodic disease or familial paroxysmal polyserositis, is an inherited auto-inflammatory disorder, which is characterized by recurrent and self-limiting episodes of fever and serositis (1, 2). The disease is characterized by severe and painful attacks of peritonitis, pleuritis, and arthritis (1, 2). Between the attacks patients are considered practically healthy. FMF is most common among people of Mediterranean area (Armenian, non-Ashkenazi Jews, Arabs, Turks) (1, 2, 3).

Shepard Siegal described FMF as a separate nosological entity in 1945 (2).

The prevalence rate of FMF varies among the Mediterranean population: 1 case per 250-1000 for Sephardic Jewish people, 1 case per 1000 for Turkish people, 1 case per 2500 for Arabic people (3). Data about the prevalence rate for Armenians is controversial. Based on the epidemiology among Armenian populations in Lebanon and Southern California FMF prevalence rate is estimated 1 case per 500 population and a gene frequency of 1:7 (3, 4). In the study of the FMF published in 1974, Khachadurian and Armenian (5) reported a minimum estimated prevalence rate of 100 per 100000 population for the 200000 Armenians in Lebanon compared with an estimated rate of 200 per 100000 population for the 20000 Armenians living in Fresno County, California (6).

According to different studies the prevalence rate among Armenians varies from 1 per 200 to 1 per 1000 (3, 7, 8). The prevalence rate of FMF among Armenians living in Yerevan is estimated 1 per 100 (7). These data can vary because of discovery of MEFV gene (1997),

which is responsible for FMF, migration of population during the recent decade and lack of studies on the epidemiology of FMF.

The onset of the disease varies among patients by age: manifestation of the disease is more frequent among young population. Among all persons with FMF, 50-60% are younger than 10 years, 80-95% are younger than 20 years, and 5-10% are older than 20 years (1, 2). FMF manifestation is rare in persons older than 40 years (1, 2, 3, 9). The disease occurs more frequently among males and according to different studies the male-female ratio ranges 1.5-2.0:1.0 (2, 3, 10).

The typical feature of FMF is the attack (paroxysm), the classic onset of which occurs without warning, although some patients may be able to detect premonitory symptoms (1, 2, 3). Disease attacks usually last 48-96 hours. The most common clinical features are the following: fever, peritoneal, pleural, pericardial, synovial symptoms and dermatologic manifestations (1, 2, 3, 11). During the attack temperature rises rapidly to 38-40°C. Temperature increases may occur before any other manifestations (3). Almost all patients with FMF experience abdominal episodes (2). Patients develop severe abdominal pain that may progress to peritonitis, resembling a surgical abdomen. Patients frequently have symptoms similar to appendicitis or cholecystitis, and they frequently have appendectomies and cholecystectomies because the abdominal episodes of FMF are not recognized properly (2). The frequency of pleural and pericardial attacks varies among different ethnic groups, 25-80% of patients reporting pleuritic episodes (1, 2, 3, 5, 7). The rate of synovial symptoms varies from 25-75% in reported studies (2, 3, 5). The episodes may resemble gout in their acute onset and intensity. Knees, ankles, and wrists are the joints most commonly affected. The joints are normal between attacks, and permanent damage usually does not occur. Arthritic symptoms tend to last several days longer than abdominal symptoms (1, 2).

One of the main steps in the FMF investigations was the discovery of the gene MEFV (MEditerranean FeVer) in 1997 (The International FMF Consortium., 1997; The French FMF Consortium., 1997), which confirm the genetic nature of the disease (2). The disease is caused by mutations in the MEFV gene, which is located on the short arm of chromosome 16 and coding the protein "pyrin" (2, 12). Nowadays no less than 29 mutations of the MEFV gene are revealed (13, 14). According to the Center of Medical Genetics for Armenians the most common MEFV mutations, namely, M694V, V726A, M680I, E148Q, F479L, R761H, M694I, are revealed in 98% of FMF patients (13). Overall carrier rate for FMF mutations is extremely high in healthy Armenian population, calculated at 0.21, or 1:5 (13).

Renal amyloidosis

Late diagnosis, misinterpretation of the FMF symptoms and inadequate treatment usually lead to complication such as renal amyloidosis, which determines the disease prognosis (1, 2, 3).

Amyloidosis is a group of diseases, which are characterized by the deposition of an abnormal fibrous protein – amyloid protein – in the extracellular spaces of different tissues and organs. There are many diseases, which cause the amyloidosis. Some of them are systematic, where amyloid is deposited in many organs; the others are localized amyloidosis, where the amyloid protein is deposited in a single organ. The amyloid protein is always derived from a normal protein precursor, which may be a serum protein, a tissue protein, a hormone or an enzyme inhibitor (14).

The amyloidosis of FMF is chemically composed of AA protein. The AA protein derives from the serum amyloid protein – SAA, an apo lipoprotein attached to HDL (14). The SAA protein is a normal serum protein. It is an acute phase protein whose plasma level may be rising following inflammation, tissue injury, pregnancy and so on (14). So the FMF-connected amyloidosis is the AA type, the same chemical type of reactive amyloidosis that

accompanies chronic infections such as tuberculosis and bronchielclasis, chronic inflammations such as rheumatoid arthritis and Crohn's disease, and certain malignant tumors (hypernephroma) and Hodgkin's disease. Amyloidosis in FMF patients, although of the AA type, has three features not observed in reactive amyloidosis associated with other disease:

a) It is very frequent in untreated FMF patients and occurs in over 90% of FMF patients of North African Jewish origin (15).
b) It appears at an early age; 90% of patients who died from amyloidosis were under 40 and 6% were under 10 (15).
c) Some FMF patients can present with renal amyloidosis in early childhood with no previous symptomatic FMF attacks. They are classified as having phenotype II FMF (14,15).

The clinical syndrome in FMF- associated renal amyloidosis, as in almost all cases of AA amyloidosis, is nephropathy, starting with proteinuria through nephrotic syndrome to renal failure (1). In majority of cases of AA amyloidosis, the renal failure develops after several years of progressive nephropathy. Patients should be treated by chronic dialysis and renal transplantation when they reach end stage renal disease (1, 14).

Heller et al. proposed that FMF and amyloidosis were two independent phenotypic expressions of the same gene. Blum et al. classified two phenotypes of the disease. In phenotype I, clinical attacks precede amyloidosis, and in phenotype II, amyloidosis is the initial or sole manifestation of the disease (9). According to the different studies two possible theories of amyloidosis development during FMF are suggested (14, 16):

1. Amyloidosis as a result of recurrent attacks.

In FMF, the recurrent inflammatory attacks are accompanied by extremely high concentrations of serum amyloid protein A (SAA), an acute-phase protein. During many

years of active disease the SAA fibers change their physical properties, which lead to amyloid deposition in various organs, mostly in kidney.

2. Genetic explanation for amyloidosis.

The presence of family history of amyloidosis has been defined as the most important risk factor in the development of amyloidosis. The researchers suggest that additional genetic factors may be operative in the development of amyloidosis (17).

AA amyloidosis may appear in FMF patients who do not suffer from febrile inflammatory attacks (phenotype II). The course of FMF is also possible without renal amyloidosis development (9, 14).

Renal amyloidosis, causing renal failure, is a frequent occurrence in patients with FMF. The prevalence of amyloidosis varied according to the geographic situation: 0% in California (Schwabe and al), 6% in Lebanon (Armenian et al) and 24% in Armenia (Ayvazian and al) (6, 15).

Armenian HK emphasized the importance of an "enrollment bias" (differences in referral pattern, in case selection, and in the sources of data) in accounting for significant variation in the frequency of different clinical manifestations in published series of FMF-associated amyloidosis (6).

According to the several studies the risk factors for amyloidosis can be summarized as early onset of the disease, male gender, frequent attacks, protracted synovitis, homozygote mutation of M694V gene (17, 18, 19, 20). The other studies have defined the family history of amyloidosis as the most important risk factor for amyloidosis development (17).

FMF and FMF-associated amyloidosis treatment

Since the 1950s, a number of different drugs have been prescribed for the treatment and/or prevention of attacks and amyloidosis. There were different antibiotics, hormones, corticosteroids, analgesics, vitamins among them (1). Colchicine has been the preferred

treatment for FMF since 1972 (15, 21). It is effective in suppressing the attacks and preventing the development of amyloidosis (21-25). Currently colchicine is the treatment of choice for FMF. Independent randomized placebo-controlled trials demonstrate colchicine's effectiveness on preventing FMF attacks (22, 23). Researchers not only reported benefit from colchicine in reducing painful attacks in FMF, but also in improving proteinuria and in preventing chronic renal failure in patients with amyloid nephropathy of FMF (16, 21).

The prognosis of AA amyloidosis in FMF is determined by the extent of amyloid at the time of diagnosis and treatment initiation and the effectiveness with which production of SAA can be suppressed by colchicine therapy (16, 26).

As researchers revealed, the early detection of the disease is very effective for the prevention of the amyloidosis development (7, 17, 16, 18, 26). Early diagnosis of FMF and daily use of 1-2 milligrams of colchicine diminish the frequency of attacks and can prevent the development of amyloidosis (7, 16, 26). So early diagnosis and adequate treatment (dose and duration) can reduce the risk of FMF-associated amyloidosis.

Most of the colchicine's side effects encountered in daily clinical practice are of gastrointestinal origin: nausea, vomiting, abdominal cramps and diarrhea that may improve with lactose-free diet. The other side effects of colchicine are very rare. They are hematological side effects (leucopenia, thrombocytopenia and hemolytic anemia), colchicine-induced myopathy, and colchicine-induced neuropathy (dyseesthesia) (27).

Objectives and Rationale for Study Design

The prevalence of FMF-associated amyloidosis in Armenia is 33.6% (2003); new amyloidosis cases are registered with prevalence 26.8%+/-2.25% per year (28). In Armenia more than half of hospitalized FMF patients with amyloidosis already have had chronic renal

failure (CRF). Among these patients, 60.6% already have had the terminal stage of CRF. Renal failure as consequence of renal amyloidosis requires not only medical assistance, but also financial resources for hemodialisis and renal transplantation, which is very rare in Armenia.

The incidence of FMF and FMF-associated amyloidosis in Armenia has remained high, especially among untreated patients. There is no epidemiologic study in Armenia connected with the evaluation of the effectiveness of colchicine therapy in preventing amyloidosis among Armenian FMF patients. The dose and duration of the colchicine treatment needed, the influence of the age at which treatment is initiated, and the effectiveness of different colchicine therapy regimens remain unclear in Armenian FMF patients. The current study was designed to examine the association between colchicine exposure and FMF-associated amyloidosis in Yerevan, Armenia. The research hypothesis was that risk of having FMF-associated amyloidosis decreases with colchicine treatment in adequate dose, with continuous/uninterrupted treatment, and with earlier age at initiation of colchicine treatment.

The case-control study design is proposed to investigate the relationship between dependent (outcome-amyloidosis) and independent variables (colchicine). The case-control study enables to assess the colchicine therapy effectiveness under routine circumstances (real life situation). Compared with experimental trials, case-control method provides estimate of colchicine effect that is more realistic than in an experimentally selected environment. On the other hand, case-control study design gives opportunity to study multiple exposures at the same time (different durations, different schemes, and different doses of used colchicine). It is expected that the results of the study will inform clinical practice on preventing and slowing down development of the renal amyloidosis by using colchicine of adequate dose and at appropriate age.

Subjects and Methods

Research question and study design

The research question was to determine whether there was an association between using colchicine, its adequate dosage (1.2 – 1.8 mg/day), its duration, mode of colchicine use and age at initiation of colchicine treatment, and the risk of developing amyloidosis in FMF patients in Yerevan, Armenia. The study was designed as case-control study with two controls for each case. The target population was Armenian patients with FMF. The FMF patients with already developed amyloidosis and FMF patients without amyloidosis were served as cases and as controls, respectively. Verification of the FMF diagnosis by genetic test is available in Armenia only by services provided by Center of Medical Genetics (CMG). The study participants were selected from the database available in the CMG. FMF patients included in the register are followed-up by the family physician and rheumatologist at the CMG. The patients with suspicion on FMF are referred to the CMG from other health care facilities for the confirmation of the diagnosis by genetic analysis. The patients willing to be followed by the CMG's physicians are registered in the center for regular examination and medical observation. FMF patients' medical record contains patients' contact information, results of genetic test, results of laboratory analyses and information regarding treatment of the patients. The study participants were selected after the review of the medical records in CMG. Approximately eight hundred FMF patients' medical records were available in the CMG. Based on the inclusion/exclusion criteria 198 participants were selected. FMF patients who developed amyloidosis within last 2 years (08/2003-08/2005) were included in the study (42 participants). From the remaining 156 FMF patients without amyloidosis, controls were selected using random number generator (RANDI command of calculator). Incident cases of amyloidosis are needed in order to address research question properly. CMG was the best source of selecting study participants, since the patients are accurately followed-up and the

first stage of amyloidosis is possible to detect. Generally, the diagnosis of amyloidosis is confirmed by renal, rectal or gingival biopsy. Since the biopsy is very rarely performed in Armenia, amyloidosis cases were selected by the protein presence in at least two sequent urine analyses, without leukocituria. After contacting with patients they were asked to come to CMG in order to perform face-to-face interview in the CMG.

Controls and cases were selected from the same health care facility in order to have a comparison group (controls) that has similar "opportunity for exposure" to colchicine as the cases.

Study population

All patients, who were genetically diagnosed with FMF from 2000 to 2005 year and were followed-up in the Center of Medical Genetics and are residents of Yerevan.

Definition of cases

FMF patients with amyloidosis developed during the period from 08/2003 to 08/2005.

Definition of controls

FMF patients without amyloidosis.

Exclusion criteria

FMF patients from marzes, not Armenian residents of Yerevan, patients with incomplete medical records and without contact information were excluded from the study.

Main Variables

The presence of amyloidosis was considered as outcome (dependant) variable of the study. Main exposure variables included current colchicine use at the time of observation, colchicine use in adequate dose/duration, age at initiation of treatment, and mode of colchicine use. Summary of the study exposure variables and their measurement scales are

presented in the Table 1. The information regarding colchicine exposure was recalled from questionnaires and was confirmed by patients' medical records. Current colchicine users at the time of observation were compared with former and never colchicine users. Adequate colchicine use was defined as using colchicine at dose 1.2 – 1.8 mg at least one year (7). For defining adequate colchicine users the index (reference) date for controls was chosen as 08/2004, since the period of amyloidosis debut was 08/2003-08/2005. So, the exposure to colchicine was compared before the date of amyloidosis debut and before the reference date, for cases and controls respectively. Adequate colchicine users were compared with non-adequate colchicine users (colchicine users at dose 0.6 – 1.0 mg and colchicine users at dose 1.2 -1.8 mg less than 1 year). Study participants were classified on mode of colchicine use as permanent users and colchicine users with interruption.

Table 1. Dependent and Exposure Variables by type and measurement scale

Variable type/name	Type	Measure
Outcome (dependant) Case status	Binary	Measured as 1 (cases) or 0 (control) group
Independent 1. Current colchicine users	Binary	Measured as 1 (Yes) or 0 (No)
2. Colchicine users in adequate dose/duration	Binary	Measured as 1 (Yes) or 0 (No)
3. Age at initiation of colchicine treatment	Continuous	Measured as numbers corresponding to the age of the colchicine treatment initiation
4. Mode of Colchicine use among current users	Binary	Measured as 1 (Permanent users) or 0 (Users with interruption)

The other independent variables included demographic, clinical and genetic characteristics of FMF patients (See Appendix 1). Demographic characteristics included study participants age and gender. Clinical characteristics included age at onset of FMF, age at FMF diagnosis, presence of the frequent attacks, presence of the abdominal and chest pain, and presence of the arthritis. The genetic characteristics involved family history of FMF,

family history of amyloidosis, specific mutations of the MEFV gene. The information regarding these characteristics was recalled from the questionnaires.

Demographical, clinical and genetic characteristics of FMF patients were collected in order to examine for potential confounders. In the original study on cloning of MEFV, the investigators noted that patients bearing the M694V mutation experienced a more severe disease and a higher rate of amyloidosis, compared with those carrying V726A (2). More recent studies confirmed the observation that patients homozygous for M694V mutations present with early onset of FMF, experience more joint involvement and frequent attacks, need a higher dose of colchicine to control their attacks and are more prone to develop amyloidosis (17-20).

Ethical Considerations

The FMF patients were informed of the aim and purpose, methods, anticipated benefits of the study by oral consent form (See Appendix 2, 3). Anonymous and voluntary character of participation was explained to all participants. Only the study investigator had access to the information provided by participants. No personal identification information was collected. The study was approved by the Institutional Review Board/Committee on Human Research (IRB) of the American University of Armenia. Permission for the implementation of the study was received from the head of Center of Medical Genetics Sarkissian T.F.

Sample size

Taking into account the values of odds ratios from the previous cohort studies (16, 21), the proportion of Armenian FMF patients with amyloidosis who uses colchicine at least for one year ($p = 0.48$), and power equal to 0.8, the number of cases and controls were calculated. The following formula was used for sample size calculations (calculations by

STATA for different values are presented in the Appendix 4). It was decided to include 2 controls for each case in order to increase power. Since 42 cases were available, it was decided to include as much FMF patients with amyloidosis as possible.

$$n = \frac{\left\{z_{1-a/2}\sqrt{2P_2(1-P_2)} + z_{1-b}\sqrt{P_1(1-P_1)+P_2(1-P_2)}\right\}^2}{(P_1-P_2)^2}$$

$$P_1 = \frac{(OR)P_2}{(OR)P_2 + (1-P_2)}$$ P_1 = proportion exposed in cases

P_2 = proportion exposed in controls

Data Collection. Description of the instrument

Interviewer administered questionnaire was used for collecting data regarding colchicine exposure among cases and controls (See Appendix 5, 6). The data collection was performed in August, 2005. The study instrument was created on the basis of the questionnaire for proposal "Determination of Attack Precipitating Factors for familial Mediterranean fever" (Yelena Amirkhanyan, Master of Public Health Thesis Project). The questionnaire consists of demographic questions, questions on the disease history, therapy questions. Demographic questions included such characteristic of the participants as sex, age, information about relatives suffered from FMF and/or renal amyloidosis. Disease history part contained questions connected with the disease specific characteristics and the features of the disease course (the onset of the attacks, their duration and frequency, the clinical form of the attacks, the involvement of the joints, specific mutations of MEFV gene). All questionnaire items regarding therapy were developed by the investigator. This part included questions regarding colchicine treatment: age at initiation, dose, duration, and skipping patterns. The questionnaire was pre-tested among 4 FMF patients and some changes were made in wording

to avoid misunderstanding. Both phone (15 interviews) and face-to-face (84 interviews) interviews were used for data collection.

Data Analysis

Information from cases and controls was entered into SPSS 11 data screen. Logistic regression was performed using STATA 7. Statistical analysis included descriptive statistics (frequency tables) and simple and multiple logistic regression. All statistical tests were two-sided. The differences between patients with and without amyloidosis were evaluated with chi-squared analysis, calculation of odds ratios (OR) with 95% confidence intervals (CI), or the t-test, as appropriate. An unconditional multivariate logistic regression analysis was performed to control for confounding by age, gender, onset, family history of amyloidosis, specific mutations of MEFV gene, where appropriate.

Results

The data were collected on 33 cases and 66 controls. Non-response rate (refusal rate) was 6% among cases (2 out of 35 patients refused to participate), and 6% among controls (4 out of 70 patients refused to participate).

Demographical, clinical and genetic characteristics of the FMF patients with and without amyloidosis are summarized in Table 2.

Table 2. Distribution of Demographical, Clinical and Genetic Characteristics by Case-Control Status

Variable	**Cases:** FMF patients with amyloidosis (n = 33)	**Controls:** FMF patients without amyloidosis (n = 66)	**p-value**	**OR**	**95 % CI**
Demographical					
Gender, N (%)					
Male	**21 (63.6%)**	**28 (42.4%)**			
Female	**12 (36.4%)**	**38 (57.6%)**	**0.047** [1]	**1.05**	**1.01-1.09**
Age (years; mean +/- SD)	**33.9 +/- 13.1**	**28.1 +/- 10.0**	**0.017** [1]		
Clinical					
Age at onset of FMF (years; mean +/- SD)	10.8 +/- 8.4	11.8 +/- 9.6	0.612		
Age at diagnosis of FMF (years; mean +/- SD)	29.4 +/- 13.7	25.9 +/- 9.2	0.126		
Time between disease onset and diagnosis (years; mean +/- SD)	**18.6 +/- 12.2**	**14.0 +/- 9.5**	**0.044** [1]		
Time between disease onset and amyloidosis debut (for cases) and observation time (for controls) [Disease duration] (years; mean +/- SD)	**22.1 +/- 13.0**	**16.3 +/- 9.3**	**0.012** [1]		
Frequent attacks (more than 10 per year), N (%)	26 (78.8%)	44 (67.7%)	0.254	1.77	0.66-4.74
Arthritis, N (%)	26 (78.8%)	40 (61.5%)	0.090	2.32	0.88-6.14
Abdominal pain, N (%)	31 (93.9%)	56 (87.5%)	0.333	2.21	0.44-11.08
Chest pain, N (%)	23 (69.7%)	48 (75.0%)	0.577	0.77	0.30-1.95
Genetic					
Family history of FMF					
• sister / brother	5 (15.2%)	13 (20.9%)	0.493	0.67	0.22-2.09
• other relative	13 (39.4%)	29 (44.6%)	0.622	0.81	0.34-1.89
Family history of amyloidosis	**12 (39.4%)**	**12 (18.8%)**	**0.031** [1]	**2.82**	**1.10-7.20**
Mutation of MEFV gene: M694V, N (%)	28 (84.85%)	43 (70.49%)	0.129	2.34	0.78-7.04
No M694V mutation	5 (15.15%)	18 (29.51%)		1.00 [2]	
1 M694V mutation	15 (45.45%)	34 (55.74%)	0.435	1.59	0.50-5.08
2 M694V mutations	**13 (39.39%)**	**9 (14.75%)**	**0.013** [1]	**5.20**	**5.20-19.18**

[1] Statistically significant variables (p-value < 0.05)
[2] Reference group

Variable	**Cases:** FMF patients with amyloidosis (n = 33)	**Controls:** FMF patients without amyloidosis (n = 66)	**p-value**	**OR**	**95 % CI**
V726A, N (%)	9 (27.3%)	29 (47.5%)	0.059	0.42	0.17-1.03
No V726A mutation	24 (72.73%)	32 (52.46%)	*		
1 V726A mutation	9 (27.27%)	27 (44.26%)			
2 V726A mutations	0	2 (3.28%)			
M680I, N (%)	11 (33.3%)	22 (36.1%)	0.791	0.89	0.36-2.16
No M680I mutation	22 (66.7%)	39 (63.9%)	*		
1 M680I mutation	10 (30.3%)	20 (32.8)			
2 M680I mutations	1 (3.0%)	2 (3.3%)			
F479L, N (%)	0	3 (4.9%)	*		
M694I, N (%)	0	1 (1.6%)	*		
R761H, N (%)	3 (9.1%)	7 (11.5%)	0.721	0.77	0.19-3.21

** For these variables the data were insufficient to obtain interpretable results*

Cases and controls differed significantly with respect to sex and age. Among cases the males dominated compared with controls (OR=1.05; 95% CI 1.01-1.09). Mean age of cases was higher in contrast to that of controls (33.9 +/- 13.1 years and 28.1 +/- 10.0 years, respectively).

The mean age at onset of FMF attacks in patient with amyloidosis was 10.8 +/- 8.4 years, while it was 11.8 +/- 9.6 years in patients without amyloidosis. Those with amyloidosis were younger at the time of disease onset, however not significantly (p-value > 0.05). There was no significant difference in age at the time FMF was diagnosed among cases and controls, which were 29.4 +/- 13.7 years and 25.9 +/- 9.2 years, respectively.

The period between disease onset and diagnosis was longer in patients with amyloidosis than in other FMF patients, which were 18.61 +/- 12.22 years and 14.03 +/- 9.52 years, respectively. The FMF patients with amyloidosis were diagnosed later compared with those without amyloidosis (p-value < 0.05). The mean duration of the disease in cases was 22.12 +/- 13.01, while it was 16.30 +/- 9.30 in controls. The duration of the disease was significantly longer among cases compared with controls (p-value < 0.05). The study participants also were compared in terms of disease different characteristics and the MEFV

gene mutations. The statistically significant association was found with the family history of amyloidosis and the risk of developing amyloidosis (OR=2.82; 95% CI 1.10-7.20). According to the revealed results presence of two M694V mutations in the MEFV gene versus no M694V mutation increases the risk of amyloidosis (OR=5.2; 95% CI 1.41-19.18).

The distribution of cases and controls regarding colchicine exposure (current users, age at initiation of colchicine treatment, total time of colchicine used, users with interruption) were summarized in the Table 3.

Table 3. Unadjusted Odds Ratios and 95% Confidence Intervals for Colchicine Exposure

Variable	**Cases:** FMF patients with amyloidosis (n = 33)	**Controls:** FMF patients without amyloidosis (n = 66)	**p-value**	**OR**	**95 % CI**
Colchicine exposure, N (%) Current users Ever/never users	 26 (78.8%) 7 (21.2%)	 59 (89.4%) 7 (10.6%)	 0.153	 0.44	 0.14-1.38
Colchicine users in adequate dose/duration (1.2-1.8 mg/ day at least 12 months), N (%) **Adequate users** **Non-adequate users**	 **13 (48.2%)** **14 (51.8%)**	 **44 (73.3%)** **16 (26.7%)**	 **0.022** [1]	 **0.34**	 **0.13-0.87**
Age at initiation of colchicine treatment (years, mean +/- SD)	**32.0 +/- 13.0**	**27.2 +/- 9.9**	**0.049** [1]	**1.04**	**1.00-1.08**
Duration of colchicine used from starting date to amyloidosis debut (cases) and to index date (controls) (months, mean +/- SD)	25.9 +/- 18.7	21.9 +/- 20.5	0.562		
Mode of Colchicine use among current users **Permanent users** **Users with interruption**	 **12 (44.4%)** **15 (55.6%)**	 **45 (75.0%)** **15 (25.0%)**	 **0.006** [1]	 **0.27**	 **0.10-0.70**
Duration of interruption (among users with interruption) • up to 3 months • from 3 to 6 months • from 6 to 9 months • from 9 to 12 months	 0 8 (53.33%) 4 (26.67%) 3 (20.00%)	 9 (60.00%) 4 (26.67%) 1 (6.67%) 1 (6.67%)	 *		

[1] Statistically significant variables (p-value < 0.05)

* For these variables the data were insufficient to obtain interpretable results

The results have shown that 78.8 % of all cases and 89.4 % of all controls used colchicine at the time of observation at different dose (0.6 – 1.8 mg dose). Only 9.1% of controls mentioned that they have never used colchicine in contrast to 18.2% of cases. The risk of developing FMF-associated amyloidosis is lower in case of adequate colchicine users versus non-adequate users (OR=0.34; 95% CI 0.13-0.87). Mean age of initiation of colchicine treatment was 27.2 +/- 9.9 for controls compared with 32.0 +/ 13.0 for cases. The risk of developing FMF-associated amyloidosis decreases with earlier age at initiation of colchicine treatment (p-value < 0.05). The risk of developing FMF-associated amyloidosis is lower in case of permanent colchicine users versus colchicine users with interruption (OR=0.27; 95% CI 0.10-0.70).

According to the results of the study there was no statistically significant effect of using colchicine currently and total time of colchicine used on the developing FMF-associated amyloidosis. However, the colchicine current use demonstrated the protective effect on developing amyloidosis.

The data were insufficient to obtain interpretable result from simple logistic regression for duration of interruption while using colchicine.

The effects of each colchicine exposure variables on risk of developing amyloidosis were adjusted for different confounding variables (age, gender, disease onset, where appropriate) (Model 1). The genetics characteristics of the disease (family history of amyloidosis, M694V mutation) were also put into the model (Model 2). Log Likelihood Ratio test was performed in order to determine if adding genetics improve fit of the model for the data comparing Models 1 and 2. Possible interactions between different factors were examined and no significant interactions were found. The characteristics of different models were summarized in the Table 4.

Table 4. Results of Logistic Regression Models of Colchicine Exposure-Incident Amyloidosis Association

Exposure Variables	Crude Model, OR; 95% CI	Adjusted Model 1 OR; 95% CI	Adjusted Model 2 OR; 95% CI	Log likelihood test (Model 2 compared to Model 1)
Colchicine exposure				$Chi^2 = 12.37$ p = 0.0021
Current users	0.44 (0.14-1.38)	0.27 (0.08-0.95)†	0.20 (0.05-0.86) ††	
Ever/never users	1.00	1.00	1.00	
Colchicine users in adequate dose/duration (1.2-1.8mg/ day at least 12 months)				$Chi^2 = 8.44$ p = 0.0148
Adequate users	0.34 (0.13-0.87)	0.33 (0.12-0.93) †	0.45 (0.15–1.33) ††	
Non-adequate users	1.00	1.00	1.00	
Age at initiation of Colchicine treatment	1.04 (1.00-1.08)	1.06 (1.01-1.11)‡	1.05 (0.99–1.10) ‡‡	$Chi^2 = 15.27$ p = 0.0005
Mode of Colchicine use among current users				$Chi^2 = 9.56$ p = 0.0084
Permanent users	0.27 (0.10-0.70)	0.29 (0.10-0.82) †	0.14 (0.04-0.53) ††	
Users with interruption	1.00	1.00	1.00	

† Adjusted for age and gender
†† Adjusted for age, gender, family history of amyloidosis and M694V
‡ Adjusted for onset of the FMF and gender
‡‡ Adjusted for onset of the FMF, gender, family history of amyloidosis and M694V

The risk of developing amyloidosis among FMF patients is lower in case of current colchicine users versus non-current (ever/never colchicine users) colchicine users controlling for age and gender (OR = 0.27; 95% CI 0.08 - 0.95). According to the results of likelihood ratio test, the best fitting (parsimonious) model for current colchicine users is model 2. Based on the result of the model, risk of developing FMF-associated amyloidosis is lower in case of current colchicine users versus non-current colchicine users controlling for age, gender and genetic factors (family history of amyloidosis and M694V gene) (OR = 0.20; 95% CI 0.05 - 0.86).

Risk of developing FMF-associated amyloidosis is 67% (1-0.33) lower in case of adequate colchicine users versus non-adequate colc hicine users after controlling for age and gender (OR=0.33; 95% CI 0.12-0.93). Controlling for gender and onset of the FMF, the risk of developing FMF-associated amyloidosis is higher in case of older age at initiation of colchicine treatment versus earlier age at initiation of colchicine treatment. Risk of developing FMF-associated amyloidosis is 6% higher in case of one year later start of colchicine use after controlling for gender and onset of FMF attacks.

There is a protective effect of permanent versus interrupted use of Colchicine on risk of amyloidosis, after controlling for age and gender (OR = 0.29; 95% CI 0.10-0.82). The protective effect was strengthening after controlling for age, gender, family history of amyloidosis and M694V mutation (OR = 0. 14; 95% CI 0.04 - 0.53).

Discussion and Recommendations

The current study aim was to evaluate the effectiveness of colchicine therapy (age at initiation, use in adequate dose, its duration, and skipping pattern) in preventing amyloidosis in Armenian FMF patients. The main findings demonstrated by the study were statistically significant associations (protective effect) between colchicine exposure and the risk of developing FMF-associated amyloidosis. According to the results of the study the important

points were that adequate use and uninterrupted use of colchicine as well as earlier age at initiation of treatment were protective of amyloidosis development in FMF patients. They remain protective after controlling for age and gender. In fact for current use and uninterrupted use they become even more protective after controlling more fully for the confounders (age, gender, family history of amyloidosis, gene M694V).

The findings of the current study regarding an effectiveness of colchicine therapy were consistent with previous reports from other studies that examined the relationship between colchicine exposure and amyloidosis in FMF patients with other ethnicity. Previous research illustrated the effectiveness of colchicine by the two-third reduction in prevalence of amyloidosis in cohort study with 7-11 years of follow-up in Jewish FMF patients (16, 21).

Biological explanation of the effect of treatment on amyloidosis

In vitro colchicine has shown to inhibit the secretion of serum amyloid protein – SAA, which is the precursor of amyloid A protein (14, 16). If the pathogenesis of amyloidosis involved a simple progression of overproduction of SAA, which leads to the amyloid fibrils formation, the effectiveness of colchicine could be understood. However, other researchers stated that amyloidosis of FMF is genetically determined (17). The current study shows the strong association between family history of amyloidosis and the risk of developing FMF-associated amyloidosis (OR=2.82; 95% CI 1.10 - 7.20). Colchicine provides protection for developing amyloidosis not only in patients whose attacks relieved by the colchicine, but also in those whose attacks not alleviated with drug (16). It seems that prevention of amyloidosis by colchicine cannot be fully explained by suppression of the attacks or suppression of secretion amyloid precursor. However, cohort studies following the preliminary observations confirmed that the drug was not only effective in preventing the attacks but also secondary amyloidosis (16, 21). A quarter of century later, colchicine is still the only available treatment.

The current study had several limitations. One of the limitations was the small sample size of the current study. The results of logistic regression were not interpretable for some variables, which could be explained with small number of observations. It might be recommended to increase the sample size in a future study. One of the acceptable ways of increasing the sample size and getting more genaralisable results is to recruit FMF patients from other health care facilities, including from marzes.

Another possible problem is connected with recall bias, which is typical for case-control studies. However, the information regarding colchicine exposure (age at initiation, dose, duration) was confirmed by the patients' medical records.

The other potential problem with study could be the misclassification bias by disease status. Persistent proteinuria (at least in two sequent urine analysis with the absence of leukocyturia) was considered to indicate amyloidosis and possibility that this led to overdiagnosis of amyloidosis should be considered. Assumption that all cases of proteinuria will be due to amyloidosis, may lead to the misclassification bias, since the possible controls may be classified wrongly as cases. The mean age of the selected FMF-associated amyloidosis cases was 33.9 +/- 13.1. In populations of young patients, persistent proteinuria has proved to be the best indication of renal amyloidosis (1, 16).

The problem with study instrument was that reliability and validity of the instrument were not determined. The other potential source of bias might be different methods of interviews: majority of the interviews were conducted face-to-face and several interviews by phone.

The cost of the genetic test for verification of the FMF diagnosis is about 40 US dollars, which is expensive for general Armenian population. This could affect negatively the generalizability of the study results, since the FMF patients from CMG do not represent the whole FMF patients in Armenia. The other concern regarding generalizability of the study

was the inclusion in the current study only FMF patients from Yerevan. Thus the generalizability of the study was limited.

Response rate of the current study was 94%. The non-response bias was not an issue since the reasons for refusal unrelated to exposure status. The main reasons for refusal to participate include patients unwilling to complete the interview and to speak about their disease.

The study demonstrated evidence that colchicine treatment is effective in preventing amyloidosis in FMF patients. Earlier start of initiation of colchicine treatment by FMF patients needs to be emphasized by the physicians. Regular continuous lifelong therapy with colchic ine in adequate dose 1.2-1.8 mg per day has to be recommended to all FMF patients in order to prevent them from amyloidosis development.

Current study was the first epidemiological study in Armenia to show the effectiveness of the colchicine treatment in preventing amyloidosis. One of the strengths of the study is available information on genotypes (MEFV gene mutations) of FMF patients and therefore ability to adjust for them.

The results of the study analysis demonstrated the need for further and comprehensive investigations for evaluation of the effectiveness of colchicine therapy in preventing FMF-associated amyloidosis. For further research cohort study for the evaluation of the effectiveness of colchicine therapy among Armenian FMF patients can be suggested. This gives opportunity to measure exposure more precisely, like person-years or months of use and establish temporal relationship between exposure-colchicine and outcome-amyloidosis. The other possible future research might be connected with evalua tion of the effectiveness of colchicine among FMF patients without family history of amyloidosis; in this case the differences between cases and controls give the pure effect of therapy on amyloidosis.

Another interesting research might be related to study FMF patients who had not develop amyloidosis by for example, the age of 40. Assessment and evaluation of their disease characteristics, specific mutations of MEFV gene and colchicine effects will help in understanding the amyloidosis development patterns in FMF patients.

It is recommended to increase sample size in a future study. Inclusion of patients from marzes will improve the generalizability of the study.

The results of current study and future comprehensive research will help to decrease the morbidity and mortality of FMF-associated amyloidosis and improve FMF patients' quality of life.

References

1. Sohar E, Gafni J, Pras M, Heller H, Familial Mediterranean fever. A survey of 470 cases and review of the literature. *American Journal of Medicine*. 1967 Aug; 43:227-53

2. Samuels J, Aksantijevich I, Torosyan Y, Centola M, Deng Z, Sood R, et al. Familial Mediterranean Fever at the Millennium. Clinical spectrum, ancient mutations, and a survey of 100 American Referrals to the National Institute of Health. *Medicine (Baltimore)*. 1998 Jul; 77(4):268-97

3. Meyerhoff J. Mediterranean Fever, Familial. *eMedicine Journal*, December 5 2000, Volume 1, Number 12. [on-line] [cited 2004 Sep 20]; Available from: URL: http://www.emedicine.com/med/topic1410.htm

4. Rogers DB, Shohat M, Petersen GM, Bickal J, Congleton J, Schwabe AD, et al. Familial Mediterranean fever in Armenians: autosomal recessive inheritance with high gene frequency. *Am J Med Genet*. 1989 Oct; 34(2):168-72.

5. Khachadurian AK, Armenian HK. Familial paroxysmal polyserositis (familial Mediterranean fever) incidence of amyloidosis and mode of inheritance. *Birth Defects*. 1974;10:62-6

6. Armenian HK. Enrollment bias and variation in clinical manifestations: a review of consecutive cases of familial paroxysmal polyserositis. *J Chronic Dis*. 1983;36:209-12

7. Ayvazyan Al.A. Treatment of Familial Mediterranean Fever by colchicine. Dissertation of medical sciences candidate. Erevan, 1988

8. Shohat M, Danon Y, Rotter J. Familial Mediterranean Fever: Analysis of Inheritance and Current Linkage Data. Am J Med Gen 1992, 44:183-188.

9. Armenian HK, Shaar KH. Epidemiologic observations in Familial paroxysmal polyserositis. Epidemiologic reviews. 1986, vol. 8, pp. 106-116

10. Sarksyan T, Ajrapetyan H, Hovanessyan, Z, Beglaryan A, Astvacatryan V. Patterns of FMF morbidity in modern Armenian population. (Materials of the III International Conference on FMF), [on-line] [cited 2004 Sep 24]; Available from: URL: http://congres.igh.cnrs.fr/FMF2002

11. Schwabe AD, Reters RS. Familial Mediterranean fever in Armenians. Analysis of 100 cases. *Medicine (Baltimore)*, 1974; 55 (6) 453-62

12. Orbach H, Chetrit E. Familial Mediterranean fever - a review and update. *Minerva Med*. 92 (6):421-430, 2001.

13. Ajrapetyan A, Hovanessyan Z, Shahsuvaryan G, Sarkisian T. The spectrum of MEFV mutations in Armenian population (Materials of the III International Conference on FMF), [on-line] [cited 2004 Sep 24]; Available from: URL: http://congres.igh.cnrs.fr/FMF2002

14. Pras M. Amyloidosis of Familial Mediterranean fever (Materials of the II International Conference on FMF). 2000

15. Pras M, Bronshpigel N, Zemer D and Gafni J. (1982) Variable incidence of amyloidosis in familial Mediterranean fever among different ethnic groups. Johns Hopkins Med J 150:22-6

16. Zemer D, Pras M, Sohar E, Modan M, Gabili S, Gafni J. Colchicine in the prevention and treatment of the amyloidosis of familial Mediterranean fever. *New England Journal of Medicine*. 1986 Apr 17; 314(16):1001-5

17. Saatci U, Ozen S, Ozdemir A, Bakkaloglu A, Besbas N, Topaloglu R. Familial Mediterranean fever in children: report of a large series and discussion of the risk and prognostic factors of amyloidosis. *Eur J Pediatr* (1997) 156: 619-623

18. Ben-Chetrit E. Familial Mediterranean fever (FMF) and renal AA amyloidosis--phenotype-genotype correlation, treatment and prognosis. *J Nephrol.* 16(3):431-4, 2003.

19. Cefle A, Kamili S, Sayarlioglu M, Inans M, Ocal L, Aral O, et al. A comparison of clinical findings of familiar Mediterranean fever patients with and without amyloidosis.

20. Papazyan, M.; Sarkisyan,T; Babloyan, A; Sanamyan, A; Leumman, E; Sarkissian, A Who is at risk to develop renal amyloidosis in familial Mediterranean fever(FMF)? (Materials of the III International Conference on FMF), [on-line] [cited 2004 Sep 24]; Available from: URL: http://congres.igh.cnrs.fr/FMF2002

21. Gabili S, Zemer D, Pras M, Aviram A, Sohar E, Gafni J. The prevention of amyloidosis in familial Mediterranean fever with colchicine. *Proc Eur Dial Transplant Assoc* Eur Ren Assoc. 1985; 21:709-11

22. Goldstein RC, Schwabe AD. Prophylactic colchicine therapy in familial Mediterranean fever. A controlled, double-blind study. *Annals of Internal Medicine*. 1974 Dec; 81(6): 792-4

23. Dinarello CA, Wolff SM, Goldfinger SE, Dale DC, Alling DW. Colchicine therapy for familial Mediterranean fever. A double-blind trial. *New England Journal of Medicine*. 1974 Oct 31; 291(18):937-7

24. Wolff SM, Dinarello CA, Dale DC, Goldfinger SE, Alling DW. Colchicine therapy of familial Mediterranean fever. Trans Assoc Am Physicians. 1974;87:186-94

25. Wright DG, Wolff SM, Fauci AS, Alling DW. Efficacy of intermittent colchicine therapy in familial Mediterranean fever. *Annals of Internal Medicine*. 86:162-165, 1977

26. A. Oner, O. Erdogan, G. Demircin, M. Bulbul, and L. Memis. Efficacy of colchicine therapy in amyloid nephropathy of familial Mediterranean fever. *Pediatr.Nephrol.* 18 (6):521-526, 2003.

27. Tunca M. Colchicine update. (Materials of the III International Conference on FMF), [on-line] [cited 2004 Sep 24]; Available from: URL: http://congres.igh.cnrs.fr/FMF2002

28. Simonyan, A. Ch. Clinical and genetic risk factors for amiloidosis development in patients with periodic disease. Dissertation of medical sciences candidate. Moscow, 2003.

List of Potential Journals for Publication

1. Journal of the American Society of Nephrology

2. American Journal of Public Health

3. Clinical and Experimental Nephrology

APPENDIX 1. Research Variables by Name and Measurement Scale

Variable type/name	Type	Measure
Independent variables Age	Continuous	Measured as numbers corresponding to patients' age
Gender Age at onset	Binary Continuous	Measured as 1 (Male) or 0 (Female) Measured as numbers corresponding to age at FMF onset
Age at diagnosis	Continuous	Measured as numbers corresponding to age at FMF diagnosis
Number of patients with frequent attacks (more than 10 per year)	Binary	Measured as 1 (Yes) or 0 (No)
Number of patients with arthritis	Binary	Measured as 1 (Yes) or 0 (No)
Number of patients with chest pain	Binary	Measured as 1 (Yes) or 0 (No)
Number of patients with abdominal pain	Binary	Measured as 1 (Yes) or 0 (No)
Family history of FMF • sister / brother • other relative	Binary	Measured as 1 (Yes) or 0 (No)
Family history of amyloidosis	Binary	Measured as 1 (Yes) or 0 (No)
Mutations of MEFV gene M694V V726A M680I F479L E148Q R761H M694I ***Independent exposure variables*** Current colchicine users	Binary Binary	Measured as 1 (Yes) or 0 (No) Measured as 1 (Yes) or 0 (No)
Colchicine users in adequate dose/duration	Binary	Measured as 1 (Yes) or 0 (No)
Age at initiation of colchicine treatment	Continuous	Measured as numbers corresponding to the age of the colchicine treatment initiation
Mode of Colchicine use among current users	Binary	Measured as 1 (Permanent users) or 0 (Users with interruption)
Duration of interruption (among users with interruption)	Ordinal	Measured as 1 (up to 3 months) 2 (from 3 to 6 months) 3 (from 6 to 9 months) 4 (from 9 to 12 months)

APPENDIX 2. English Version of Study Consent Form

American University of Armenia
College of Health Sciences
Master of Public Health Program
CONSENT FORM

Title of Research Project
Evaluation of the effectiveness of colchicine therapy in preventing renal amyloidosis in patients with Familial Mediterranean Fever in Yerevan, Armenia. Case-control study.

Explanation of Research Project

The Public Health department of AUA is conducting a study regarding Familial Mediterranean Fever. The purpose of the study is to obtain the information about the effectiveness of colchicine therapy in preventing renal amyloidosis in patients with FMF. You have been chosen to participate in the study, because you have Familial Mediterranean Fever. We appreciate your participation in this study and your responses are highly valuable for us.

Procedures

Your name was obtained from the records maintained by the Center of Medical Genetics. All that is required of you is the one time completion of a questionnaire. The time required for the completion of the questionnaire is approximately 15-20 minutes.

Risks/Discomforts

This study involves no risk for participants.

Benefits
You will not directly benefit from participation in this study. The information provided by you may help to evaluate the effectiveness of the treatment in patients with FMF which could benefit other FMF patients.

Confidentiality

Your name, surname and telephone number will be kept confidential. A code number will be assigned to the questionnaire. No individual can be identified from the information provided in the questionnaire. Your personal information will not appear in the report. Only combined data will be reported. Your responses will be accessible only in the Public Health department in the American University of Armenia.

Voluntariness

It is your decision whether to participate in the study or not. You have the right to stop providing information at any time you wish or skip any question you consider inappropriate.

Your refusal to participate in the study or your decision to withdraw from that at any time will not influence your job or study.

Whom to contact

The name of the researcher is Maria Sevoyan, a second year student of Public Health Department in American University of Armenia. You may ask her any question regarding the study.
You should ask the person in charge any questions you may have about this research. You should ask him/her questions in the future if you do not understand something that is being done. The researchers will tell you anything new they learn that they think will affect you. The results of the study will be publicly available. The report will maintain in the Public Health Reference Library in American University of Armenia.

If you want to talk to anyone about this research study you should call the person in charge of the study: **[Yelena Amirkhanian] at [phone number: (3741) 51 25 68 / e-mail: yamirkh@auaam].**
If you want to talk to anyone about the research study because you feel you have not been treated fairly or think you have been hurt by joining the study you should contact the Public Health Department, American University of Armenia at **(3741) 51 2568**.

Thank you very much for your participation.

APPENDIX 3. Armenian Version of Study Consent Form

Ð³Û³ëï³ÝÇ²Ù»ñÇÏÛ³ÝÐ³Ù³Éë³ñ³Ý
Ð³Ýñ³ÛÇÝ²éáÕç³å³ÑáõÃÛ³Ý´³ÅÇÝ
ÎñÃ³Ï³Ý ì»ñ³Ý³ÛÙ³Ý ÊáñÑáõñ¹
Ð³Ù³Ó³ÛÝ³·Çñ

Ð»ï³½áïáõÃÛ³Ý³Ýí³ÝáõÙÁ
ÎáÉËÇóÇÝÇ ¿ý»Ïï ÇíáõÃÛ³Ý·Ý³Ñ³ïáõÙÁ »ñÇÏ³ÙÝ»ñÇ³ÙÇÉáÇ¹á½Ç Ï³ÝË³ñ·»ÉÙ³Ý Ñ³ñóáõÙ` ºñ¨³ÝáõÙ µÝ³Ïíáõ å³ñµ»ñ³Ï³ÝÑÇí³Ý¹áõÃÛ³ÙµÑÇí³Ý¹Ý»ñÇ Ùáï:

Ð»ï³½áïáõÃÛ³Ýµ³ó³ïñáõÃÛáõÝÀ

Ð³Û³ëï³ÝÇ²Ù»ñÇÏÛ³ÝÐ³Ù³Éë³ñ³ÝÇ Ð³Ýñ³ÛÇÝ²éáÕç³å³ÑáõÃÛ³Ý Ìñ³·ñáõÙ ÁÝ¹·ñÏí³Íáõë³ÝáÕáõÑÇÝ Çñ³Ï³Ý³óÝáõÙ ¿Ñ»ï³½áïáõÃÛáõÝ å³ñµ»ñ³Ï³Ý ÑÇí³Ý¹áõÃÛ³Ýí»ñ³µ»ñÛ³É: ¸ñ³Ýå³ï³ÏÝ¿·Ý³Ñ³ï»ÉÏáÉËÇóÇÝÇ ¿ý»Ïï ÇíáõÃÛáõÝÁ »ñÇÏ³ÙÝ»ñÇ³ÙÇÉáÇ¹á½Ç Ï³ÝË³ñ·»ÉÙ³Ý Ñ³ñóáõÙ` ºñ¨³ÝáõÙ µÝ³Ïíáõ å³ñµ»ñ³Ï³ÝÑÇí³Ý¹áõÃÛ³ÙµÑÇí³Ý¹Ý»ñÇ Ùáï:
¸áõù ÁÝïñí»É»ù Ù³ëÝ³Ïó»Éáõ ïíÛ³É Ñ»ï³½áïáõÃÛ³ÝÁ, áñáíÑ»ï¨¹áõù ÑÇí³Ý¹ »ù å³ñµ»ñ³Ï³ÝÑÇí³Ý¹áõÃÛ³Ùµ:
Ð»ï³½áïáõÃÛ³ÝÁ Ò»ñ Ù³ëÝ³ÏóáõÃÛáõÝÁß³ï ³ñÅ»ù³íáñ¿: Ò»ñ ÏáÕÙÇó ïñ³Ù³¹ñí³Íï»Õ»ÏáõÃÛáõÝÝ»ñÁß³ï û·ï³Ï³ñ ÏÉÇÝ»Ý ³Ûë Ñ»ï³½áïáõÃÛ³Ý Ñ³Ù³ñ:

ÀÝÃ³ó³Ï³ñ·

Ò»ñ³ÝáõÝÁ í»ñóí»É¿´ÅßÏ³Ï³Ý¶»Ý»ïÇÏ³ÛÇ Î»Ýïñáõ Ù å³Ñå³Ýí³Íµ ÅßÏ³Ï³Ý ·ñ³éáõÙÝ»ñÇó: Ð³Ù³Ó³ÛÝáõÃÛ³Ý¹»åùáõÙ, Ù³ëÝ³ÏóÇó å³Ñ³Ýçíáõ Ù¿ÙÇ³ÛÝ Ù»Ï³Ý·³Ù å³ï³ëË³Ý»É ïñ³Ù³¹ñí³Í Ñ³ñó³Ã»ñÃÇÏÇÑ³ñó»ñÇÝ: Ð³ñó³Ã»ñÃÇÏÇ Ñ³ñó»ñÇÝ å³ï³ëË³Ý»Éáõ ³ÝÑñ³Å»ß ï Å³Ù³Ý³ÏÁ Ï³½Ùáõ Ù¿ 15– 20 ñáå»ÛÇó áã ³í»É:

ì ï³Ý·/²ÝÑ³ñÙ³ñáõÃÛáõÝ

Ø³ëÝ³Ïó»Éáí Ñ»ï³½áïáõÃÛ³ÝÁ` ¹áõù áã ÙÇ íï³Ý·Çã»ù »ÝÃ³ñÏíáõÙ:
Ð³ñó³Ã»ñÃÇÏÁ ãÇ å³ñáõÝ³ÏáõÙ Ñ³ñó»ñ, áñáÝù Ï³ñáÕ»Ý³ÝÑ³ñÙ³ñáõÃÛ³Ý å³ï×³é¹³éÝ³É Ò»ñ Ñ³Ù³ñ:

Þ³ÑáõÛÃ/ú·áõ ï

¸áõù³ÝÙÇç³Ï³Ýû·áõïã»ù áõÝ»Ý³Ñ» ï³½áïáõÃÛ³ÝÁ Ò»ñ Ù³ëÝ³ÏóáõÃÛáõÝÇó: ´³Ûó, ³ÛÝáõ³Ù»Ý³ÛÝÇí, Ò»ñ ÏáÕÙÇó ïñ³Ù³¹ñí³Íï»Õ»ÏáõÃÛáõÝÝ»ñÁ Ïû·Ý»Ý·Ý³Ñ³ï»É ÏáÉËÇóÇÝÇ ¿ý»Ïï ÇíáõÃÛáõÝÁ »ñÇÏ³ÙÝ»ñÇ³ÙÇÉáÇ¹á½Ç Ï³ÝË³ñ·»ÉÙ³Ý Ñ³ñóáõÙ` ºñ¨³ÝáõÙ µÝ³Ïíáõ å³ñµ»ñ³Ï³ÝÑÇí³Ý¹áõÃÛ³ÙµÑÇí³Ý¹Ý»ñÇ Ùáï:
Ð»ï³½áïáõÃÛ³ÝÁÝÃ³óùáõÙ Ó»ñùµ»ñí³Íï»Õ»ÏáõÃÛáõÝÝ»ñÁ Ïû·Ý»ÝÏ³ÝË³ñ·»É»É »ñÇÏ³ÙÝ»ñÇ³ÙÇÉáÇ¹á½Ç½³ñ·³óáõÙÁ å³ñµ»ñ³Ï³ÝÑÇí³Ý¹áõÃÛ³ÙµÑÇí³Ý¹Ý»ñÇ Ùáï, áñÁû·áõï Ïµ»ñÇ å³ñµ»ñ³Ï³ÝÑÇí³Ý¹áõÃÛ³Ùµ ³ÛÉÑÇí³Ý¹Ý»ñÇ:

¶ ³ Õ ï ÝÇáõÃÛáõÝ

Ð» ï ³½á ï áõÃÛáõÝÁ ãÇ å ³ Ñ ³ ÝçáõÙ Ò»ñ ³ ÝÓÝ ³ Ï ³ Ý ï íÛ³ ÉÝ»ñÁ, ÇÝãåÇëÇÝ »Ý ³ ÝáõÝ ¨ ³½· ³ ÝáõÝÁ, Ñ»é ³ Ëáë ³ Ñ ³ Ù ³ ñÁ ¨ Ñ ³ ëó»Ý: æ»ñ ³ ÝáõÝÁ ãÇ ·ñ ³ Ýóí»Éáõ Ñ ³ ñó ³ Ã»ñÃÇÏáõÙ, Ñ ³ ñó ³ Ã»ñÃÇÏÇÝ ï ñí»Éáõ ¿ Ñ ³ Ù ³ ñ Ï ³ Ù Ïá¹: Ò»ñ ³ ÝÓÁ ãÇ Ï ³ ñáÕ áñáßí»É Ñ ³ ñó ³ Ã»ñÃÇÏÇ ï íÛ³ ÉÝ»ñÇ ÑÇÙ ³ Ý íñ ³ : Î ³ å ³ Ñáí íÇ Ò»ñ ÏáÕÙÇó ï ñ ³ Ù ³ ¹ñí ³ Í µáÉáñ ï »Õ» ÏáõÃÛáõÝÝ»ñÇ · ³ Õ ï ÝÇáõÃÛáõÝÁ: ´ áÉáñ ï íÛ³ ÉÝ»ñÁ Ý»ñÏ ³ Û ³ óí»Éáõ »Ý ³ Ù÷á÷Çã ï íÛ³ ÉÝ»ñÇ Ò¨áí: Ò»ñ ÏáÕÙÇó ï ñ ³ Ù ³ ¹ñí ³ Í µáÉáñ ï »Õ» ÏáõÃÛáõÝÝ»ñÁ Ïû· ï ³ ·áñÍ í»Ý ÙÇ ³ ÛÝ Ð ³ Û ³ ë ï ³ ÝÇ ² Ù»ñÇÏÛ ³ Ý Ð ³ Ù ³ Éë ³ ñ ³ ÝÇ Ð ³ ë ³ ñ ³ Ï ³ Ï ³ Ý ²éáÕç ³ å ³ ÑáõÃÛ ³ Ý µ ³ ÅÝÇ ÏáÕÙÇó í»ñçÝ ³ Ï ³ Ý í»ñÉáõÍáõÃÛáõÝ Ï ³ ï ³ ñ»Éáõ Ýå ³ ï ³ Ïáí:

Î ³ Ù ³ íáñáõÃÛáõÝ

Ð» ï ³½á ï áõÃÛ ³ ÝÁ Ò»ñ Ù ³ ëÝ ³ ÏóáõÃÛáõÝÁ ÉÇáíÇÝ Ï ³ Ù ³ íáñ ¿: ¸ áõù Çñ ³ íáõÝù áõÝ»ù Ññ ³ Å ³ ñí»Éáõ Ù ³ ëÝ ³ ÏóáõÃÛáõÝÇó Ñ» ï ³½á ï áõÃÛ ³ Ý ó ³ ÝÏ ³ ó ³ Í å ³ ÑÇÝ: ¸ áõù Ï ³ ñáÕ »ù Ññ ³ Å ³ ñí»É ³ ÛÝ Ñ ³ ñó»ñÇÝ å ³ ï ³ ëË ³ Ý»Éáõó, áñáÝù Ï · ï Ý»ù½· ³ ÛáõÝ Ï ³ Ù ³ ÝÑ ³ ñÙ ³ ñ: Ð» ï ³½á ï áõÃÛ ³ ÝÁ Ù ³ ëÝ ³ Ïó»Éáõ Ò»ñ ³ ÝÑ ³ Ù ³ Ó ³ ÛÝáõÃÛáõÝÁ áã ÙÇ µ ³ ó ³ ë ³ Ï ³ Ý Ñ» ï ¨ ³ Ýù ãÇ áõÝ»Ý ³ ¨ áã ÙÇ Ó¨áí ãÇ ³ Ýñ ³ ¹ ³ éÝ ³ Ò»ñ ³ ßË ³ ï ³ ÝùÇ Ï ³ Ù áõëÙ ³ Ý íñ ³ :

àõÙ Ñ» ï Ï ³ åÝí»É

Ð» ï ³½á ï áÕÇ ³ ÝáõÝÁ Ø ³ ñÇ ³ ê¨áÛ ³ Ý ¿, Ð ³ Û ³ ë ï ³ ÝÇ ² Ù»ñÇÏÛ ³ Ý Ð ³ Ù ³ Éë ³ ñ ³ ÝÇ áõë ³ ÝáÕ: ¸ áõù Ï ³ ñáÕ »ù Çñ»Ý ó ³ ÝÏ ³ ó ³ Í Ñ ³ ñó ï ³ É Ñ» ï ³½á ï áõÃÛ ³ Ý í»ñ ³ µ»ñÛ ³ É: ¸ áõù Ï ³ ñáÕ »ù ¹ÇÙ»É ë ï áñ¨ ÝßÍ ³ Í å ³ ï ³ ëË ³ Ý ³ ï áõ ³ ÝÓÇÝ ó ³ ÝÏ ³ ó ³ Í Ñ ³ ñóáí, áñÁ Í»ñ ³ µ»ñíáõÙ ¿ Ñ» ï ³½á ï áõÃÛ ³ ÝÁ: ¸ áõù Ï ³ ñáÕ »ù Ý ³ ¨ ¹ÇÙ»É Ýñ ³ Ý Ñ» ï ³ · ³ ÛáõÙ, »Ã» ÇÝã-áñ µ ³ Ý ³ ÝÑ ³ ë Ï ³ Ý ³ ÉÇ ÉÇÝÇ Ò»ñ Ñ ³ Ù ³ ñ: ä ³ ï ³ ëË ³ Ý ³ ï áõ ³ ÝÓÁ Ï å ³ ï ³ ëË ³ ÝÇ Ò»ñ µáÉáñ Ñ ³ ñó»ñÇÝ ¨ ÏÑ ³ Û ï ÝÇ ³ ÛÝ Ýáñ ë ï ³ ó ³ Í ï »Õ» ÏáõÃÛáõÝÝ»ñÁ, áñáÝù, Ýñ ³ Ï ³ ñÍÇùáí, Ï ³ ñáÕ »Ý ³½¹»óáõÃÛáõÝ áõÝ»Ý ³ É Ò»ñ ³ éáÕçáõÃÛ ³ Ý íñ ³ :

ºÉ»Ý ³ ² ÙÇñË ³ ÝÛ ³ Ý (3741) 51 25 68

àõëáõÙÝ ³ ëÇñáõÃÛ ³ Ý ï íÛ ³ ÉÝ»ñÁ ÉÇÝ»Éáõ »Ý Ñ ³ ë ³ Ý»ÉÇ Ñ ³ ë ³ ñ ³ ÏáõÃÛ ³ ÝÁ ¨ å ³ Ñí»Éáõ »Ý Ð ³ Û ³ ë ï ³ ÝÇ ² Ù»ñÇÏÛ ³ Ý Ð ³ Ù ³ Éë ³ ñ ³ ÝÇ êáÕÇÏÛ ³ ÝÇ ³ Ýí ³ Ý ·ñ ³ ¹ ³ ñ ³ ÝáõÙ: ÐÝ ³ ñ ³ íáñ ¿ Ý ³ ¨ ¹ñ ³ Ýù ï å ³ ·ñí»Ý Ù ³ ëÝ ³ ·Ç ï Ï ³Ý ³ Ùë ³ ·ñáõÙ: ºÃ» Ò»ñ Ï ³ ñÍÇùáí, Ò»ñ Ñ» ï í ³ ñí»É »Ý áã ³ ñ ³ ¹ ³ ñ ³ óÇáñ»Ý Ï ³ Ù íÝ ³ ë/íÇñ ³ íáñ ³ Ýù »ù ë ï ³ ó»É` Ù ³ ëÝ ³ Ïó»Éáí ³ Ûë Ñ» ï ³½á ï áõÃÛ ³ ÝÁ, ¸ áõù Ï ³ ñáÕ »ù ¹ÇÙ»É Ð ³ Û ³ ë ï ³ ÝÇ ³ Ù»ñÇÏÛ ³ Ý Ñ ³ Ù ³ Éë ³ ñ ³ Ý Ñ» ï ¨Û ³ É Ñ»é ³ Ëáë ³ Ñ ³ Ù ³ ñáí` (374 1) 51 26 71.

ºÃ» ¸ áõù Ñ ³ Ù ³ Ó ³ ÛÝ »ù Ù ³ ëÝ ³ Ïó»É ³ Ûë Ñ» ï ³½á ï áõÃÛ ³ ÝÁ, ËÝ¹ñ»Ù, ë Ïë»Ýù:

ÞÝáñÑ ³ Ï ³ ÉáõÃÛáõÝ Ò»ñ Ù ³ ëÝ ³ ÏóáõÃÛ ³ Ý Ñ ³ Ù ³ ñ:

APPENDIX 4. Estimated sample size for two-sample comparison of proportions (not-equal sample sizes n1 / n2 = 0.5). STATA results

Test Ho	Assumptions	Estimated required sample sizes
$p_1 = p_2$, where p_1 is the proportion in cases and p_2 is the proportion in controls	OR = 0.28 alpha = 0.05 (two-sided) power = 0.80 ratio ($n_1 / n_2 = 0.5$) $p_1 = 0.48$ $p_2 = 0.77$	$n_1 = 37$ $n_2 = 74$
$p_1 = p_2$, where p_1 is the proportion in cases and p_2 is the proportion in controls	OR = 0.24 alpha = 0.05 (two-sided) power = 0.80 ratio ($n_1 / n_2 = 0.5$) $p_1 = 0.48$ $p_2 = 0.78$	$n_1 = 34$ $n_2 = 68$
$p_1 = p_2$, where p_1 is the proportion in cases and p_2 is the proportion in controls	OR = 0.3 alpha = 0.05 (two-sided) power = 0.80 ratio ($n_1 / n_2 = 0.5$) $p_1 = 0.48$ $p_2 = 0.75$	$n_1 = 42$ $n_2 = 84$
$p_1 = p_2$, where p_1 is the proportion in cases and p_2 is the proportion in controls	OR = 0.4 alpha = 0.05 (two-sided) power = 0.80 ratio ($n_1 / n_2 = 0.5$) $p_1 = 0.48$ $p_2 = 0.69$	$n_1 = 64$ $n_2 = 128$
$p_1 = p_2$, where p_1 is the proportion in cases and p_2 is the proportion in controls	OR = 0.35 alpha = 0.05 (two-sided) power = 0.80 ratio ($n_1 / n_2 = 0.5$) $p_1 = 0.48$ $p_2 = 0.73$	$n_1 = 50$ $n_2 = 100$

APPENDIX 5. English Version of the Questionnaire

QUESTIONNAIRE

Evaluation of the Effectiveness of Colchicine Treatment in Preventing Renal Amyloidosis in Patients with Familial Mediterranean fever in Yerevan, Armenia

ID Number*: ___ / ___ ___ ___

*** The coding for ID Number**

Digit 1	Disease ID Number[1]
Digit 2-3-4	Respondent's number in the list

[1] The coding for Disease ID Number

Familial Mediterranean Fever without renal amyloidosis	0
Familial Mediterranean Fever with renal amyloidosis	1

DEMOGRAPHIC QUESTIONS

1) Gender:
   1. Male
   2. Female

2) Year of birth: __ __ __ __ (YYYY) _____ (age)

3) Are you currently employed?
   1. Employed
   2. Unemployed
   3. Student
   4. Other _____

4) Do you have brother (s) or sister (s)?

1. Yes
2. No Go to *QUESTION 6*

5) Do/Does they/he/she suffer from the Familial Mediterranean Fever?

1. Yes
2. No
3. Don't know

6) Does/did any of your other relatives also suffer from the FMF?

1. Yes
2. No
3. Don't know

7) Does/did any of your relatives suffer/die from the kidney disease?

1. Yes
2. No
3. Don't know

(Space is provided for drawing the respondent's pedigree)

QUESTIONS ON DISEASE HISTORY

8) At what age did you have the first attack? _____

9) At what age was your disease "Familial Mediterranean Fever" first medically diagnosed?

_____

10) Is your disease proved by genetic analysis?

1. Yes _____ (*specify mutation*)
2. No
3. Don't know

11) What type was your first attack?

1. Abdominal (acute pain in abdomen)
2. Pleuritis (acute pain in chest)
3. Synovitis (joint pain)
4. Skin manifestation (skin eruption)
5. Renal amyloidosis first (kidney affection)
6. Mixed (combination of symptoms) _____
7. Other*(specify)* _____

12) What kind of symptoms are added during the disease progression and when?

1. Abdominal pain _____
2. Chest pain _____
3. Joint pain _____
4. Skin manifestation/skin eruption _____
5. Renal amyloidosis _____
6. Mixed (combination of symptoms) _____
7. Other*(specify)* _____ _____

13) During first two-three years of the disease (before treatment with colchicine), how many attacks do you have per year?

1. Less than 10 per year
2. 10 per year
3. More than 10 per year
4. Other *(specify)* _____

14) What is the mean duration of the attack?

1. Several hours
2. 1-2 day
3. 3 days
4. More than 3 days
5. Other (specify) _____

15) Do you have arthritis/arthralgia (joint pain)?

1. Yes
2. No Go to *QUESTION 18*

16) During the joint attacks what symptoms are revealed?

1. Arthralgia
2. Edema How many joints _____
3. Erythema How many joints _____

17) What kind of joint attacks do you have?

1. Acute (attacks last from 12 to 72 hours)
2. Subacute (Attacks last up to week)
3. Protracted (attacks last up to months)
4. Other *(specify)* _____

18) Do you receive any treatment?

1. No
2. Yes Go to *QUESTION 32*

19) What kind of treatment do you receive?

1. Painkillers (like “Anal gin”, “Baralgin”)
2. Non-steroid anti-inflammatory drugs (like “Voltaren”, “Aspirin”)
3. Steroid anti-inflammatory drugs (like “Prednizolon”)
4. Oral contraceptives
5. Colchicine
6. Other *(specify)* _____

20) Do you take Colchicine currently?

1. Yes Go to *QUESTION 22, 24*
2. No Go to *QUESTION 21*

21) Have you ever take Colchicine?

1. Yes Go to *QUESTION 22*
2. No Go to *QUESTION 32*

22) When did you start to use Colchicine? _____*(at what age)*

23) When did you stop to use Colchicine? (HELP. *How many days, weeks, months, years before?)*

_____

24) On average how often do you take/did you take Colchicine?

1. Only during/at the beginning of the attacks
2. Every day regardless the attacks
3. Every day and additional tablets during/at the beginning of the attacks
4. Not regular *(specify)* _____
5. Other *(specify)* _____

25) At what dose do you take/did you take Colchicine? _____

26) How many months do you take/did you take Colchicine?

_____ *(specify in months)*

(If for different time different dose used)

_____ Dose _____ Duration *(specify in months)*

_____ Dose _____ Duration *(specify in months)*

_____ Dose _____ Duration *(specify in months)*

_____ Dose _____ Duration *(specify in months)*

27) During the period of Colchicine taking, is Colchicine taken permanently, or with interruption?

1. Permanently Go to *QUESTION 30*
2. With interruption Go to *QUESTION 29*

28) On average, how many months do/did the interruption last? (HELP. *How many days, weeks, months, years?)*

_____

29) Does the treatment with Colchicine reduce the frequency of attacks?

1. Yes Go to *QUESTION 30*
2. No Go to *QUESTION 31*
3. Other *(specify)* _____

30) During the treatment with Colchicine, on average, how many attacks do you have per year?

1. No attacks Go to *QUESTION 32*
2. Less than 10 per year
3. 10 per year
4. More than 10 per year
5. Do not know
6. Other *(specify)* _____

31) During the treatment with Colchicine is the intensity of the attacks reduced?

1. Yes
2. No
3. Do not know
4. Other *(specify)* _____

32) Have you ever been told that you have (proteinuria) protein in your urine (during the attack free period)?

1. Yes
2. No **The interview is finished.**
3. Do not know **The interview is finished.**

33) At what age did you first have protein in your urine?

_____ *(specify age)*

The interview is finished.
Thank you for your cooperation.

APPENDIX 6. Armenian Version of the Questionnaire

Ð²ðò²Â°ðÂÆÎ

ä³ñµ»ñ³Ï³ÝÑÇí³Ý¹áõÃÛ³ÙµÑÇí³Ý¹Ý»ñÇÙáï»ñÇÏ³ÙÝ»ñÇ³ÙÇÉáÇ¹á½Ç

½³ñ·³óÙ³ÝÏáÉËÇóÇÝáíÏ³ÝË³ñ·»ÉÙ³Ýí»ñ³µ»ñÛ³ÉÑ³ñóáõÙ

î²ð´°ð²ÎØ²Ü Ð²Ø²ð* ___/___ ___ ___

***î³ñµ»ñ³ÏÙ³ÝÑ³Ù³ñÇÍ³ÍÏ³·Çñ**

ÂÇí 1 **Âí»ñ 2-3-4**	ÐÇí³Ý¹áõÃÛ³Ý ï³ñµ»ñ³ÏÙ³ÝÑ³Ù³ñÁ[1] Ð³ñóíáÕÇ Ñ³Ù³ñÁ óáõó³ÏáõÙ

[1]ÐÇí³Ý¹áõÃÛ³Ý ï³ñµ»ñ³ÏÙ³ÝÑ³Ù³ñÁ

ä³ñµ»ñ³Ï³Ý ÑÇí³Ý¹áõÃÛáõÝ ³é³Ýó»ñÇÏ³ÙÝ»ñÇ ³ÙÇÉáÇ¹á½Ç	0
ä³ñµ»ñ³Ï³Ý ÑÇí³Ý¹áõÃÛáõÝ µ³ñ¹³ó³Í »ñÇÏ³ÙÝ»ñÇ ³ÙÇÉáÇ¹á½áí	1

ÄàÔà ì ð¸²¶ð²Î²Ü î ì Ú²ÈÜ°ð

1) ê»é
   1. ²ñ³Ï³Ý
   2. Æ·³Ï³Ý

2) Ò»ñ ÍÝÝ¹Û³Ý ÃÇíÁ __ __ __ __ _____
   ï³ñÇ ï³ñÇùÁ

3) Ü»ñÏ³ÛáõÙë ³ßË³ ïáõ±Ù Ï³Ù ëáíáñáõ±Ù »ù:
   1. ²ßË³ ïáõÙ »Ù
   2. â»Ù ³ßË³ ïáõÙ
   3. êáíáñáõÙ »Ù
   4. ²ÛÉ_____

4) øáõÛñ»ñ, »Õµ³ÛñÝ»ñ áõÝ»±ù:
   1. ²Ûá
   2. àã **²Üò°ø** *Ð³ñó 6*

5) Ü ³ (Ýñ ³ Ýù) ï ³ é ³ åáõÙ »±Ý å ³ ñµ»ñ ³ Ï ³ Ý ÑÇí ³ Ý ¹áõÃÛ ³ Ùµ:

1. ²Ûá
2. àã
3. â·Ç ï »Ù

6) Ò»ñ ³½. ³ Ï ³ ÝÝ»ñÇó áñ¨Çó» ³ÛÉ Ù»ÏÁ ï ³ é ³ åáõ±Ù ¿ å ³ ñµ»ñ ³ Ï ³ Ý ÑÇí ³ Ý ¹áõÃÛ ³ Ùµ:

1. ²Ûá
2. àã
3. â·Ç ï »Ù

7) Ò»ñ ³½. ³ Ï ³ ÝÝ»ñÇó áñ¨Çó» Ù»ÏÁ áõÝÇ/áõÝ»ó»±É ¿ »ñÇÏ ³ Ù ³ ÛÇÝ ÑÇí ³ Ý ¹áõÃÛáõÝ *(Ù ³ Ñ ³ ó»É ¿ "³½á ï Çó", »ñÇÏ ³ Ù ³ ÛÇÝ ³ Ë ï ³ Ñ ³ ñáõÙÇó):*

1. ²Ûá
2. àã
3. â·Ç ï »Ù

(ï ³ ñ ³ ÍáõÃÛáõÝÁ Ý ³ Ë ³ ï »ë í ³ Í ¿ ÑÇí ³ Ý ¹Ç ï áÑÙ ³ Í ³ éÁ ÝÏ ³ ñ»Éáõ Ñ ³ Ù ³ ñ)

Ð²ðòºð ÐÆì²Ü¸àôÂÚ²Üä²îØàôÂÚ²Ü ìºð²´ºðÚ²È

8) à±ñ ï³ñÇùáõÙ ï»ÕÇ áõÝ»ó³í ³é³çÇÝ Ýáå³Ý: _____

9) à±ñ ï³ñÇùáõÙ ³é³çÇÝ ³Ý·³Ù Ò»½Ùá ï ³Ëï áñáßí»ó¦å³ñµ»ñ³Ï³Ý ÑÇí³Ý¹áõÃÛáõÝ§

_____

10) ²ñ¹Ûá±ù Ò»ñ ÑÇí³Ý¹áõÃÛáõÝÁ Ñ³ëï³ï í³±Í ¿¦·»Ý»ï ÇÏ³Ï³Ý ³Ý³ÉÇ½Ç» û·ÝáõÃÛ³Ùµ:

1. ²Ûá _____ *(Ýß»É Ùáõï³óÇ³Ý)*
2. àã
3. â·Çï»Ù

11) Æ±Ýã ï ÇåÇ¿ñ³é³çÇÝ Ýáå³Ý:

1. àñáí³ÛÝ³ÛÇÝ ó³í»ñ (ëáõñ ó³í»ñ áñáí³ÛÝÇ ßñç³ÝáõÙ)
2. Îñíù³í³Ý¹³Ï³ÛÇÝ ó³í»ñ (ëáõñ ó³í»ñ Ïñíù³í³Ý¹³ÏÇ ßñç³ÝáõÙ)
3. Ðá¹³ÛÇÝ ó³í»ñ (ó³í»ñ Ñá¹»ñÇ ßñç³ÝáõÙ)
4. Ø³ßÏÇ ³Ëï³Ñ³ñáõÙ/ó³Ý³íáñáõÙ
5. ²ÙÇÉáÇ¹á½ (»ñÇÏ³ÙÝ»ñÇ ³Ëï³Ñ³ñáõÙ)
6. Ê³éÁ (³Ëï³ÝÇßÝ»ñÇ Ñ³Ù³ÏóáõÃÛ³Ùµ) _____
7. ²ÛÉ, Ýß»ù _____

12) ÐÇí³Ý¹áõÃÛ³Ý½³ñ·³óÙ³Ý ÁÝÃ³óùáõÙ Ç±Ýã ëÇÙå ï áÙÝ»ñ/Ýß³ÝÝ»ñ ³í»É³ó³Ý¨»±ñµ:

1. àñáí³ÛÝ³ÛÇÝ ó³í»ñ _____
2. Îñíù³í³Ý¹³Ï³ÛÇÝ ó³í»ñ _____
3. Ðá¹³ÛÇÝ ó³í»ñ _____
4. Ø³ßÏÇ ³Ëï³Ñ³ñáõÙ/ó³Ý³íáñáõÙ _____
5. ²ÙÇÉáÇ¹á½ (»ñÇÏ³ÙÝ»ñÇ ³Ëï³Ñ³ñáõÙ) _____
6. Ê³éÁ (³Ëï³ÝÇßÝ»ñÇ Ñ³Ù³ÏóáõÃÛ³Ùµ) _____
7. ²ÛÉ, Ýß»ù _____ _____

13) ÐÇí ³Ý¹áõÃÛ³Ý ëï½µÝ³Ï³Ý ßñç³ÝáõÙ (³é³çÇÝ »ñÏáõ/»ñ»ù ï³ñí³ ÁÝÃ³óùáõÙ – ÙÇÝã»õ ÏáÉËÇóÇÝÇ û·ï³·áñÍáõÙÁ) ÙÇçÇÝáõÙ ù³±ÝÇ Ýáå³ »ù áõÝ»ó»É Ù»Ï ï³ñí³ ÁÝÃ³óùáõÙ:

1. 10 ùÇã Ù»Ï ï³ñí³ ÁÝÃ³óùáõÙ
2. 10 Ù»Ï ï³ñí³ ÁÝÃ³óùáõÙ
3. 10 ³í»ÉÇ Ù»Ï ï³ñí³ ÁÝÃ³óùáõÙ
4. â·Çï»Ù
5. ²ÛÉ _____

14) àñù³±Ý¿ ÉÇÝáõÙ ÙÇçÇÝáõÙ Ýáå³ÛÇ ï¨áÕáõÃÛáõÝÁ:

1. ØÇ ù³ÝÇ Å³Ù
2. 1-2 ûñ
3. 3 ûñ
4. 3 ûñÇó ³í»É
5. ²ÛÉ _____

15) Ðá¹³µáñµ»ñ (Ñá¹»ñÇ ³Ëï³Ñ³ñáõÙ) áõÝ»Ýáõ±Ù »ù:

1. ²Ûá
2. àã **²Üò°ø** *Ð³ñó 18*

16) Ðá¹»ñÇ ³Ëï³Ñ³ñáõÙÝ»ñÇ Å³Ù³Ý³Ï¹Çï íáõÙ ¿

1. ò³í Ñá¹Ç/Ñá¹»ñÇ ßñç³ÝáõÙ:
2. ²Ûï áõó Ñá¹Ç/Ñá¹»ñÇ ßñç³ÝáõÙ: ø³ÝÇ± Ñá¹áõÙ _____
3. Î³ñÙñáõÃÛáõÝ Ñá¹Ç/Ñá¹»ñÇ ßñç³ÝáõÙ: ø³ÝÇ± Ñá¹áõÙ _____

17) Æ±Ýã ïÇåÇ Ñá¹³µáñµ»ñ (Ñá¹»ñÇ ³Ëï³Ñ³ñáõÙ) »ù áõÝ»ÝáõÙ:

1. êáõñ (12 – 72 Å³Ù ï¨áÕáõÃÛ³Ùµ)
2. °ÝÃ³ëáõñ (ÙÇÝã¨ Ù»Ï ß³µ³Ã ï¨áÕáõÃÛ³Ùµ)
3. °ñÏ³ñ³ï¨ (ï¨áõÙ »Ý³ÙÇëÝ»ñ)
4. ²ÛÉ, Ýß»ù _____

18) ¸áõù ëï³Ýáõ±Ù/ëï³ó»É »ù áñ¨¿ µáõÅáõÙ å³ñµ»ñ³Ï³Ý ÑÇí³Ý¹áõÃÛ³Ý Ï³å³ÏóáõÃÛ³Ùµ:

1. ²Ûá
2. àã _____ **²Üò°ø** *Ð³ñó 32*

19) Æ±Ýã µáõÅáõÙ »ù ë ï ³ ÝáõÙ/ë ï ³ ó»É å ³ ñµ»ñ ³ Ï ³ Ý ÑÇí ³ Ý¹áõÃÛ ³ Ý Ï ³ å ³ ÏóáõÃÛ ³ Ùµ:
(Üß»É ÑÝ ³ ñ ³ íáñ µáÉáñ ï ³ ñµ»ñ ³ ÏÝ»ñÁ).

1. ò ³ í ³ ½ñÏáÕÝ»ñ (ûñÇÝ ³ Ï` ³ Ý ³ É·ÇÝ, µ ³ ñ ³ É·ÇÝ)
2. àã ÑáñÙáÝ ³ ÛÇÝ Ñ ³ Ï ³ µáñµáù ³ ÛÇÝ ÙÇçáóÝ»ñ (ûñÇÝ ³ Ï` µñáõý»Ý, ÇÝ¹áÙ» ï ³ óÇÝ, íáÉ ï ³ ñ»Ý)
3. ÐáñÙáÝ ³ É Ñ ³ Ï ³ µáñµáù ³ ÛÇÝ ÙÇçáóÝ»ñ (ë ï »ñáÇ¹Ý»ñ, ûñÇÝ ³ Ï` å ñ»¹ÝÇ½áÉáÝ)
4. Ð ³ Ï ³ µ»ÕÙÝ ³ íáñÇã Ñ ³ µ»ñ
5. **ÎáÉËÇóÇÝ**
6. ² ÛÉ, Ýß»ù _____

20) ¸ áõù û·ï ³ ·áñÍáõ±Ù »ù ÏáÉËÇóÇÝ Ý»ñÏ ³ ÛáõÙë:

1. ² Ûá _____ **² Üò°ø** *Ð ³ ñó 22, Ð ³ ñó 24*
2. àã _____ **² Üò°ø** *Ð ³ ñó 21*

21) ¸ áõù »ñµ¨Çó¿ û·ï ³ ·áñÍ»±É »ù ÏáÉËÇóÇÝ:

1. ² Ûá _____ **² Üò°ø** *Ð ³ ñó 22*
2. àã _____ **² Üò°ø** *Ð ³ ñó 32*

22) °±ñµ »ù ëÏë»É û·ï ³ ·áñÍ»É ÏáÉËÇóÇÝ: _____ *(áñ ï ³ ñÇùáõÙ)*

23) °±ñµ »ù ¹ ³ ¹ ³ ñ»óñ»É ÏáÉËÇóÇÝÇ û·ï ³ ·áñÍáõÙÁ: *(áñù ³ Ý Å ³ Ù ³ Ý ³ Ï ³ é ³ ç;* **ú·Ý»É** *áñù ³ ±Ý ûñ, ß ³ µ ³ Ã, ³ ÙÇë, ï ³ ñÇ ³ é ³ ç)*

_____

24) ØÇçÇÝáõÙ á±ñù ³ Ý Ñ ³ × ³ Ë »ù/¿Çù û·ï ³ ·áñÍáõÙ ÏáÉËÇóÇÝ:

1. ØÇ ³ ÛÝ Ýáå ³ Ý»ñÇ ëÏ½µáõÙ / ÁÝÃ ³ óùáõÙ
2. ² Ù»Ý ûñ ³ ÝÏ ³ Ë Ýáå ³ ÛÇ ³ éÏ ³ ÛáõÃÛáõÝÇó
3. ² Ù»Ý ûñ ¨ Ñ ³ í»ÉÛ ³ É Ñ ³ µ»ñ Ýáå ³ Ý»ñÇ ëÏ½µáõÙ/ ÁÝÃ ³ óùáõÙ
4. àã Ï ³ ÝáÝ ³ íáñ _____
5. ² ÛÉ _____

25) Æ±Ýã ¹»Õ ³ ã ³ ÷áí/¹á½ ³ Ûáí »ù/¿Çù û·ï ³ ·áñÍáõÙ ÏáÉËÇóÇÝ: _____

26) à±ñù³Ý Å³Ù³Ý³Ï »ù û·ï³·áñÍáõÙ ÏáÉËÇóÇÝ: _____ *(ÝßÉ ³ÙÇëÝ»ñáí)*

(»Ã» ï³ñµ»ñ Å³Ù³Ý³Ï ï³ñµ»ñ ¹»Õ³ã³÷)

_____ ¸»Õ³ã³÷ _____ Ä³Ù³Ý³Ï *(ÝßÉ ³ÙÇëÝ»ñáí)*
_____ ¸»Õ³ã³÷ _____ Ä³Ù³Ý³Ï *(ÝßÉ ³ÙÇëÝ»ñáí)*
_____ ¸»Õ³ã³÷ _____ Ä³Ù³Ý³Ï *(ÝßÉ ³ÙÇëÝ»ñáí)*
_____ ¸»Õ³ã³÷ _____ Ä³Ù³Ý³Ï *(ÝßÉ ³ÙÇëÝ»ñáí)*

27) ÎáÉËÇóÇÝÇ û·ï³·áñÍáõÙÁ (¹»Õáñ³ÛùÁ û·ï³·áñÍÙ³Ý Å³Ù³Ý³Ï³Ñ³ï í³ÍáõÙ) ³ÝÁÝ¹Ù»±ç ¿ »Õ»É, Ã»± ÁÝ¹Ñ³ï áõÙÝ»ñáí:

1. ²ÝÁÝ¹Ù»ç _____ **²Üò°ø** *Ð³ñó 30*
2. ÀÝ¹Ñ³ï áõÙÝ»ñáí _____ **²Üò°ø** *Ð³ñó 29*

28) àñù³±Ý Å³Ù³Ý³Ïáí ¿ ÁÝ¹Ñ³ï í»É ÏáÉËÇóÇÝÇ û·ï³·áñÍáõÙÁ ***(ú·Ý»É** áñù³±Ý ûñ, ß³µ³Ã, ³ÙÇë, ï³ñÇ ÁÝ¹ÙÇçáõÙáí)* :

_____

29) ÎáÉËÇóÇÝáí µáõÅÙ³Ý ³ñ¹ÛáõÝùáõÙ Ýí³½»±É ¿ ³ñ¹Ûáù Ýáå³Ý»ñÇ Ñ³×³Ë³Ï³ÝáõÃÛáõÝÁ:

1. ²Ûá **²Üò°ø** *Ð³ñó 30*
2. àã **²Üò°ø** *Ð³ñó 31*
3. ²ÛÉ _____

30) ÎáÉËÇóÇÝáí µáõÅÙ³Ý ³ñ¹ÛáõÝùáõÙ ÙÇçÇÝáõÙ ù³±ÝÇ Ýáå³ »ù áõÝ»ÝáõÙ Ù»Ï ï³ñí³ ÁÝÃ³óùáõÙ:

1. ÀÝ¹Ñ³Ýñ³å»ë ãÏ³ **²Üò°ø** *Ð³ñó 32*
2. 10 ùÇã Ù»Ï ï³ñí³ ÁÝÃ³óùáõÙ
3. 10 Ù»Ï ï³ñí³ ÁÝÃ³óùáõÙ
4. 10 ³í»ÉÇ Ù»Ï ï³ñí³ ÁÝÃ³óùáõÙ
5. â·Ç ï»Ù
6. ²ÛÉ _____

31) Î áÉËÇóÇÝáí µáõÅÙ ³ Ý ³ ñ¹Ûáõ Ýùáõ Ù Ýí ³ ½»±É ¿ ³ ñ¹Ûáù Ýáå ³ Ý»ñÇ ÇÝ ï »ÝëÇíáõÃÛáõ ÝÁ (Ýáå ³ Ý»ñÇ ï ¨áÕáõÃÛáõ ÝÁ, ó ³ íá ï áõÃÛ ³ Ý áõÅÁ):

1. ² Ûá
2. àã
3. â·Ç ï »Ù
4. ² ÛÉ _____

32) Ò»½ Ùá ï »ñµ¨¿ Ñ ³ Û ï Ý ³ µ»ñí»±É ¿ ëåÇ ï ³ Ïáõó (µ»ÉáÏ) Ù»½áõÙ (é»ÙÇëÇ ³ ÛÇ/áã Ýáå ³ Ý»ñÇ ÁÝÃ ³ óùáõÙ):

1. ² Ûá
2. àã **Ð ³ ñó ³ ½ñáÛóÁ ³ í ³ ñ ï í ³ Í ¿**
3. â·Ç ï »Ù **Ð ³ ñó ³ ½ñáÛóÁ ³ í ³ ñ ï í ³ Í ¿**

33) à±ñ ï ³ ñÇùáõÙ ¿ ³ é ³ çÇÝ ³ Ý· ³ Ù ³ Ë ï áñáßí»É ëåÇ ï ³ Ïáõó Ç/µ»ÉáÏÇ ³ éÏ ³ ÛáõÃÛáõ ÝÁ Ù»½áõÙ (åñá ï »ÇÝáõñÇ ³ Ý): _____

Ð ³ ñó ³ ½ñáÛóÁ ³ í ³ ñ ï í ³ Í ¿:
ÞÝáñÑ ³ Ï ³ ÉáõÃÛáõÝ Ù ³ ëÝ ³ ÏóáõÃÛ ³ Ý Ñ ³ Ù ³ ñ: