The Results of Tuberculin Skin Test and the Risk of Tuberculosis in Autopsy Workers

Otopsi Çalışanlarında Tüberkülin Deri Testi ve Tüberküloz Riski

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ABSTRACT Objective: Tuberculosis (TB) is a disease that arises due to *M. tuberculosis* complex and usually involves the lungs but can also affect other organs or organ systems. The number of people who died in Turkey was 210146 in 2006 and 0.11% (n=249) of them died because of pulmonary tuberculosis. During autopsy, many microparticles (aerosols) can be generated while taking the organ out of the body, washing the body, or using the oscillating saw. If these aerosols may contain bacilli that can easily infect the health workers. Tuberculin skin test (TST) determines delayed type hypersensitivity reactions to some antigenic components of microorganism. The only sign of latent tuberculosis infection is positive TST. In our study, we aimed to calculate the exposure rate of the autopsy workers to TB bacillus by evaluating TST measurements and chest radiograms. **Material and Methods:** The study group was composed of 25 physicians and five autopsy technicians. The control group was composed of 30 physicians, residents and specialists of basic medical sciences except of Microbiology. Both groups were evaluated by TST and chest X-rays from TB point of view. We used TST reaction evaluation criteria that was suggested by Ministry of Health of Republic of Turkey while evaluating TST measurements. All measurements were analyzed statistically. **Results:** TST positivity ratio is determined as 83.3% in the study group and 33.3% in the control group. The mean of TST measurement value is determined as 13.8±4.7 mm in the study group and 8.6±2.2 mm in the control group. There were no statistically significant differences between two groups concerning age, height, marital status, occupational experience or smoking habits (p>0.05). There was a statistically significant difference between study and control groups for the TST values were (F=11.786; p=0.001). There were no statistically significant differences for gender, smoking and interaction between gender-group and smoking-group (p>0.05). Established ANOVA model (univariate ANOVA); explained the =0.903 of TST change and it was statistically significant (F=71.159; p<0.001). There was a statistically significant correlation between TST results and time spent in the autopsy room (r=0.553; p=0.002). **Conclusion:** Our study shows that autopsy workers have a high risk of TB exposure. We suggest that autopsy workers should be examined for occupational TB exposure. Furthermore, morgue buildings and autopsy rooms of our country should be designed in accordance with the international standards, and measures should be taken in this regard without delay.

Key Words: Autopsy; tuberculosis; skin tests

ÖZET Amaç: Tüberküloz (TB), *M. tuberculosis* kompleks'in neden olduğu başlıca akciğerleri tutan, ancak akciğer dışı organ ya da sistemleri de tutabilen bir hastalıktır. Türkiye'de 2006'da 210146 ölüm olayı meydana gelmiş olup bunların %0.11'i (n=249) TB nedeniyle hayatlarını kaybetmişlerdir. Otopsi sırasında organların çıkartılması, cesedin yıkanması veya elektrikli testere kullanımı sırasında ortam havasına çok sayıda aerosol karışmaktadır. Eğer bu aerosoller infekte ise sağlık personelini kolaylıkla infekte edebilirler. Tüberkülin deri testi (TDT), basilin bazı antijenik yapılarına karşı vücudun gösterdiği gecikmiş tipte hipersensitivite reaksiyonunu göstermeye yarar. Latent TB enfeksiyonunun tek bulgusu TDT pozitifliğidir. Çalışmamızda, TDT ve akciğer radyografileri ile değerlendirerek, otopsi çalışanlarının TB basiline maruz kalma oranlarını belirlemeyi amaçladık. **Gereç ve Yöntemler:** Çalışma grubu 25 hekim ve beş otopsi teknisyeninden meydana geldi. Kontrol grubu ise Mikrobiyoloji dışındaki Temel Tıp Bilimleri'nde görevli 30 uzman ve asistan hekimden oluşturuldu. Her iki grup TDT ve akciğer radyografileri ile değerlendirildi. TDT sonuçlarının değerlendirilmesi sırasında, T.C.Sağlık Bakanlığı'nın önerdiği kriterler kullanıldı. Tüm bulgular istatistiksel olarak incelendi. **Bulgular:** TDT pozitifliği oranı çalışma grubunda %83.3, kontrol grubunda %33.3 olarak saptandı. Çalışma grubunda TDT ölçüm değerleri ortalaması 13.8±4.7 mm iken kontrol grubunda 8.6±2.2 mm idi. Gruplar arasında yaş, vücut ağırlığı, evlilik durumları ve meslek süreleri açısından istatistiksel olarak fark yoktu (p>0.05). Gruplar arasında TDT değerleri açısından istatistiksel olarak fark saptandı (F= 11.786; p= 0.001). Cinsiyet, sigara içilmesi, cinsiyet-gruplar ve sigara içilmesi-gruplar arasında istatistiksel olarak fark saptanmadı (p>0.05). ANOVA modeli (univariate ANOVA) oluşturuldu; TDT değişiklikleri ve istatistiksel farklılığın doğruluğu =0.903 ile izah edildi (F=71.159; p<0.001). Otopsi salonunda geçirilen süre ile TDT sonuçları arasında istatistiksel olarak anlamlı ilişki saptandı (r= 0.553; p= 0.002). **Sonuç:** Çalışmamızın sonuçları otopsi personelinin tüberküloza maruz kalma yönünden yüksek riske sahip olduklarını göstermektedir. Otopsi çalışanlarının mesleki tüberküloza maruziyeti açısından periyodik muayenelerinin yapılması ve yaptırılması gerektiğini düşünmekteyiz. Ayrıca ülkemizdeki morg binaları ve otopsi salonlarının uluslararası standartlara uygun olarak yapılandırılmasının gerekli olduğu ve bu konuda gecikmeden önlem alınması gerektiği sonucuna varılmıştır.

Anahtar Kelimeler: Otopsi; tüberküloz; deri testleri

Tuberculosis (TB) is a disease that arises due to *M. tuberculosis* complex and usually involves lungs but also can affect other organs or organ systems. The disease develops by inhalation of bacillus-containing aerosols that are generated during coughing or sneezing by people with pulmonary or laryngeal TB. People with active pulmonary TB disease infect approximately 10-15 people each year. On the other hand, consumption of unpasteurized cow's milk can also cause TB when contaminated with M. bovis. Non-pulmonary TB is usually considered as non- infectious.[1-4]

When TB bacilli stay latent and the host has no symptoms, this is called latent TB infection (LTBI). Disease can develop in 10.0% of people infected with TB bacillus at any point of lifetime. Seventy-ninety percent of people with TB bacillus eradicate the bacillus with non-specific immune mechanisms and tuberculin skin test (TST) is negative in these people. LTBI develops in remaining 10-30% of people. LTBI is determined with TST positivity. Apparent disease develops in 5.0% of the people with LTBI infection in two years after getting infected with bacillus, and this is called primary TB. Another type of TB called either "adult type" or "post-primary TB" develops in 5.0% of remaining people at any point of lifetime.[1-8]

According to the 2009 Global TB Report of World Health Organization (WHO), at least 2 billion people have been infected with TB bacillus, which is approximately equal to one third of the world population.[3] According to this report, TB mortality was 1.7 millions (26,8 /100000), TB incidence was 9.2 millions (139/100000) and TB prevalence was 13.7 millions (206/100000) in 2007.[4]

According to "2009 Report of Turkish War with Tuberculosis", the population of Turkey is 70586256; the number of new TB cases is 17781 (25.2/100000); and total number of TB cases is 19.694 (27.9/100000). The mortality was as follows: 517 (2.83%) in new cases group, 91 (4.66%) in treated group and 608 (3.01%) in all groups in 2006.[9] According to Turkish Statistical Institute data, the number of people who died in Turkey in 2006 was 210146, and 0.11% (n=249) of them died because of pulmonary TB.[10] There are approximately 400

forensic medicine specialists in Turkey. There is no data on the exact number of forensic autopsies performed annually in Turkey.

During autopsy, many micro particles (aerosols) can be generated while taking the organ out of the body, washing the body, or during the use of oscillating saw. Oscillating saw generates 5700 particles/milliliter.[11] If these aerosols contain bacilli, they can easily infect health workers.[12]

TST determines delayed type hypersensitivity reactions to some antigenic components of microorganism.[13,14] The only sign of LTBI is positive TST.[15] Although more specific tests can be used in either healthy or immune-suppressed people (interferon-gamma release assays [IGRAs]), TST is frequently used for the diagnosis of LTBI and in epidemiological studies of TB.[16]

Bacille Calmette-Guérin (BCG) vaccine is administered and recommended for the prevention and control of tuberculosis in developing countries with high-risk. In our country, BCG vaccination is performed routinely and this is why BCG vaccination history should not be considered while evaluating TST measurements.[5,13,17]

In our study, we aimed to investigate the exposure rate of autopsy workers to TB bacillus by evaluating TST measurements and chest radiograms.

▌MATERIAL AND METHODS

Thiry-one participants were included into the study group and one of them was excluded due to subcutaneous injection and because of history of atopy. Twenty-five of the participants were physicians and five were autopsy technicians. The study group (30 participants) was composed of forensic medicine specialists, one pathologist, autopsy technicians and forensic medicine residents. Residents were at the institute where the study was held, for rotational education programs for a period of 1-6 months. TST was applied to the forensic medicine residents four months later. The control group was composed of 30 physicians, residents and specialists of basic medical sciences.

Both two groups were evaluated by TST and chest X-rays for TB. All participants were vaccina-

ted by "5 TU PPD Test Tuberculin Tween 80" intracutaneous injection by Mantoux method. The injection was applied to the flexor side of the left forearm. Induration diameter was measured perpendicular to the injection line (as perpendicular to the long axis of forearm) after 72 hours and was recorded as millimeters (mm). The test was repeated because of "booster effect" if participants had 6-9 mm reaction and no BCG scar. Age, gender, the presence of BCG scar, number of scars, smoking habits and participants' habitat in the last five years was recorded. A measurement above 15 mm was considered as a positive result in participants with a BCG scar, and a measurement above 10 mm was considered as positive for participants that had no BCG scar.[13,18,19]

We used TST Reaction Evaluation Criterion suggested by Ministry of Health of Republic of Turkey while evaluating TST measurements (Table 1).

Other influential factors on TST were evaluated by "Univariate ANOVA". Correlations between demographical data and TST were evaluated. This study was performed after the approval of ethics commitee. Informed consents were collected before the study.

STATISTICAL ANALYSIS

All measurements were analyzed by graphically and Shapiro-Wilk test was performed to test normal distribution. Descriptive statistics were shown as mean±standard deviation (for the normally distributed), median (IQR-Interquartile Range) (for the non-normally distributed), and n (%) for the categorical variables. Chi-square and likelihood ratio analysis were used in order to show compression of categorical variables (group, sex etc.). Univariate ANOVA was used in order to analyze the effect of groups, gender, smoking and their interactions on TST. For the comparison of categorical variables' effects (differences) on TST; student's t test or Mann-Whitney (non-parametric equivalent of Student's t) test was applied. The correlation between demographic variables and TST values were analyzed by Pearson or Spearman rank correlation coefficients. MS-Excel and SPSS for Win Ver. 15.0 (SPSS Inc., Chicago, IL., USA) package programs were used. P ≤0.05 was accepted as significant in statistical decisions.

RESULTS

Twenty-two (73.3%) participants were males and 8 (26.7%) were females in the study group. There were 28 (93.3%) males and two (6.7%) females in the control group. The median age was 34.0 (IQR=6.75) years in the study group and it was 34.0 (IQR=7.25) years in the control group. Ninety five percent of the study participants were living in Ankara for at least 10 years. Two (6.7%) subjects from the study group and one (3.3%) subject from the control group had no BCG scar. One subject from each group without a BCG scar were born in Germany. While 1-3 BGC scars were found in the participants of the study group, 1-4 BCG scars were found in the participants of the control group.

Among all participants' 27 (45.0%) had a family with three people, 18 (30.0%) had a family with four people, and six (10.0%) had a family with two people. In the study group, 11 (36.7%) subjects were non-smokers, two (6.7%) were ex-smokers and 17 (56.7%) were smokers. In the control group, 18 (60.0%) subjects were non-smokers, four (13.3%) were ex-smokers, and 8 (26.7%) were smokers.

Two participants of the study group had hypertension, one had hyperlipidemia, one had bronchiectasis, renal failure and epilepsy. Two participants of the control group had familial mediterranean fever (FMF), two had hypertension, one had Behcet Syndrome, and one had pneumonia complication. Other participants of both groups were healthy.

TABLE 1: TST reaction evaluation criteria in Turkey.[5,13]	
BCG Scar	**TST Measurements**
People without BCG scar*	1. 0-5 mm induration: Negative(-)
	2. 6-9 mm : Suspected, test will be repeated
	1 week later, again 6-9 mm.(-), ≥ 10 mm. (+)
	3. ≥ 10 mm : Positive (+)
People with BCG scar	1. 0-5 mm : Negative (-)
	2. 6-14 mm : Due to BCG
	3. ≥ 15 mm : Positive (+), infection

* For people without BCG scar and whose immune system is suppressed,
≥ 5 mm is accepted as (+).

There were no statistically significant differences between two groups concerning age, height, marital status, occupational experience or smoking habits (p>0.05). There was a statistically significant difference between gender and body weight (p<0.05). The mean of TST measurement value was 13.8±4.7 mm in the study group and 8.6±2.2 mm in the control group. The mean TST measurements in two groups are presented in Table 2.

Test positivity ratio was determined as 83.3% in the study group and 33.3% in the control group. TST was determined as positive in two participants without any BGC scar in the study group. The test was repeated one week later in a control group participant who had no BCG scar but a 6-9 mm induration. Test result remained negative.

There was a statistically significant difference between study and control groups for TST values (F=11.786; p=0.001). There were no statistically significant differences for gender, smoking and interaction between gender and groups, smoking and groups (p>0.05).

Established ANOVA model (univariate ANOVA); explained the =0.903 of TST change and was statistically significant (F=71.159; p<0.001) (Table 3).

The median value of BCG scar frequency was 1.0 (IQR=0.25) in the study group and 1.0 (IQR=1.00) in the control group. There were no statistically significant difference between two groups for BCG scar presence and BCG scar frequency (X2=0.357; p=0.550 and Z=0.723; p=0.470, respecti-

TABLE 2: Distribution of mean TST measurements.						
Group / Variables			n	%	$\bar{X}$	Std.Dev.
Group	Study		30	50.0	13.8	4.7
	Control		30	50.0	8.6	2.2
Sex	Study	Male	22	73.3	13.5	5.3
		Female	8	26.7	14.5	2.9
	Control	Male	28	93.3	8.7	2.2
		Female	2	6.7	7.5	0.7
Smoking	Study	Non-smoker	11	36.7	12.0	5.7
		Ex-smoker	2	6.7	16.5	4.9
		Smoker	17	56.7	14.6	3.9
	Control	Non-smoker	18	60.0	8.7	2.4
		Ex-smoker	4	13.3	9.2	2.9
		Smoker	8	26.7	8.1	1.1

TST: Tuberculin skin test.

TABLE 3: ANOVA decompressions of TST.					
Source	Type III Sum of Squares	df	Mean Square	F	p
Model	8039.627(a)	8	1004.953	71.159	<0.001
Group	166.450	1	166.450	11.786	0.001
Sex	0.643	1	0.643	0.046	0.832
Smoking	29.483	2	14.741	1.044	0.359
Group * Sex	6.411	1	6.411	0.454	0.503
Group * Smoking	42.257	2	21.128	1.496	0.233
Error	734.373	52	14.123		
Total	8774.000	60			

a: $R^2 = 0.916$ (Adjusted $R^2 = 0.903$)

*: Dependent variable: TST measurement value, Fixed factors -independent variables-: Group, sex, smoking and its interactions.

TST: Tuberculin skin test.

TABLE 4: Correlations of TST with demographic variables.				
	TST			
	Study Group		**Control Group**	
Demographic Variables	R	p	R	p
Occupational experience (m) a	0.378	0.040	0.301	0.106
CFM membership duration (m) a	0.325	0.122	N/A	N/A
Work experience at morgue department (m) a	0.158	0.405	N/A	N/A
Time spend at autopsy room (h/d) a	0.553	0.002	N/A	N/A
BCG scar frequency a	0.088	0.643	0.193	0.308
The number of the family members at home b	-0.091	0.634	-0.121	0.525

(CFM: Council of Forensic Medicine, m: month, h/d: hours/day)
a: Spearman rank correlation
b: Pearson correlation
N/A: Not Available
TST: Tuberculin skin test.

vely). Additionally there were no statistically significant relations between BCG scar frequency and TST values in the study and in the control groups (p=0.643 and p=0.308, respectively) (Table 4).

Time spent in the morgue department varied between 1 month and 24 months and the median experience of forensic medicine workers at the morgue department was determined as 24.0 (IQR=75.0) months. The median working hours in the morgue department was determined as 8.0 (IQR=0.8) hours/day. Working duration in the autopsy room changed between 1-5 hours per day.

In the autopsy workers group, a statistically significant but weak positive relation was observed between occupational experience and TST (r=0.378; p=0.040). In other words, there was a statistically significant positive and weak relation between time spent in autopsy room and TST values (r=0.553; p=0.002) (Table 4).

There was a statistically significant correlation between TST results and time spent in the autopsy room (r=0.553; p=0.002).

CONCLUSION

Autopsy workers' infection risk with *M. tuberculosis* has been known since early nineteenth century. Autopsy procedure is the most suitable and the easiest way for TB bacillus to infect people in the autopsy room. Especially while performing au-

topsy of a cases without a prior TB diagnosis, infection risk of unprotected autopsy workers is higher than other physicians who were treating the patient before death. It is suggested that occupational TB risk of morgue and related laboratory workers was 100-200 times higher than normal population, while the risk of whole hospital staff was 10.98 times higher.[11, 20-22] Almost all autopsy cases had no prior medical history data in the institute where the study held. This is the same for autopsies performed across our country.

It is reported that 40 clinic staff with negative TST had no change seen in their test results after they treated an undiagnosed TB patient, however autopsy personnel who spent three hours for autopsy of this case for had positive TST 8 weeks later although their TST were negative at the beginning. Moreover, two of these personnel had positive sputum cultures eight weeks later.[23] In a similar study, it is determined that in five of the 35 medical faculty students who attended to an autopsy of a TB patient, one hour was enough for spreading of the infection. One of these infected students had spent only 10 minutes in the autopsy room. Furthermore, it is suggested that infection risk did not vary with distance from the autopsy table.[11]

If BCG vaccination was given more than 15 years previously, it should be ignored as a cause of a current positive TST result, especially if the in-

duration is > 15 mm. It seems that a strongly positive reaction (>15 mm) is more likely to be caused by tuberculosis infection rather than the effect of previous BCG vaccination.[24] In our study, there was no difference with respect to presence of BCG scar. Fifty-seven participants who have BCG scars were vaccinated more than 15 years ago. TST result was positive in two participants in the study group who had no BCG scar, and TST result was negative in one participant in the control group who had no BCG scar. We found that prior BCG vaccination history and number of BCG scars did not effect TST measurements statistically.

Teppo et al. determined the ratio of development of active TB in pathologists as 10%, in chest disease experts as 4%, and in other clinicians as 1%.[25] In a study conducted in Japan, it was reported that TB development risk among forensic pathologists due to occupational exposition was 6-11 times more with respect to other hospital workers who were not attending to autopsies. In our study, TST positivity was found as 83.3% in the study group and 33.3% in the control group. Furthermore, it was found that TST measurement increased with the increased duration of employment and with the increased duration of stay in the autopsy room.

In our study, TST value increased as the membership experience of Council of Forensic Medicine and work experience at morgue increased. There was a weak correlation between time spent in the morgue and TST value, but a strong positive correlation was found between TST values and time spent in the autopsy. It was observed that spending more time in the autopsy room increased the size of tuberculin indurations. TST results' were found positive in two of four autopsy workers who had negative TST one year previously and in two other autopsy workers TST results were found negative like their prior TST result.

The chest X-ray is normal in the majority of people who are infected with *M. tuberculosis* and had primary infection. In cases where there is difficulty in diagnosis, computerized tomography (CT) can be preferred.[7] Bronchiectasis was diagnosed with radiographic assessment in one participant in the study group. Additionally obscured left diap-

hragmatic sinus was diagnosed in one participant in the control group. All other participants' X-rays were evaluated as normal. Furthermore, computerized chest tomography of one TST positive participant from the study group was evaluated as normal.

It is reported that although TB is very rare in Western Europe, there is a risk of contamination at autopsy.[26-28] Active tuberculosis incidence at the autopsies was 1.9% in Sweden, and 44% of these cases had no prior TB diagnosis. It was reported that this rates were 1% and 74% in Japan, respectively.[29,30]

According to the 2008 Report of Ministry of Health of Turkey Republic, it was suggested that incidence of TB disease in Turkey was 28/100,000.[31] A pulmonary tissue culture study that was held at the same time and in the same place with our study suggested that TB prevalence was 1.0% in the autopsy cases.[12] TB rate was determined as 3.0% in another study from Turkey.[32] These findings suggest that only half of the cases can be diagnosed, and undiagnosed cases live in the community unaware of their disease and they may constitute a risk for contamination of healthy people.[5,33]

TB incidence among autopsy workers is reported to be three times more when compared to general population in males, and eight times more in females.[11] In our study, there was no statistically significant difference of induration measurements with respect to gender.

Aerosols are solid and liquid particles suspended in air. It is reported that 0-210 particles/ml with speaking, 0-3500 particles/ml with coughing, 4500-1000000 particles/ml with sneezing and 5700 particles/ml with use of oscillating saw spread into the air.[7,18,34-36] Although being bone TB is rare, using an oscillation saw to cut calvarium can generate infected aerosols and toxic dust. In order to prevent generation of these aerosols and dust and to prevent them to be mixed with the air, some instruments should be used.

Centers for Disease Control and Prevention (CDC) suggests that air should be ventilated at the isolation center minimally six times in an hour.[37, 38]

For the protection of autopsy workers from TB and similar diseases originating from autopsy materials; "Biosafety Level 3" should be applied to autopsy rooms. Accordingly, autopsy room should be in a separate place from the other buildings that are used for other purposes, the air flow in the room should be from outside to inside, from ceiling to floor and from clear areas to dirty areas, undirectional and separate from other rooms. Double door system should be used and there should be an audible warning system that is working when there is a problem in the air flow. N-95, P2 or N-99 type masks and high efficiency particulate aerosol (HEPA) filtered powered air purifying respirator (PAPR) should be used. The doors and taps of the room should be automatic.[39] Furthermore work places should be sterilized with the Ultraviolet-C light at the germicidal wavelength which is 254-260 nm.[37,38]

While performing autopsy, daily clothes should be undressed, and disposable, waterproof clothes should be worn. Protective cutting resistant special gloves should be worn on the two layers of surgical gloves. Protective eyeglasses and waterproof shoes or boots should be worn.[37,38,40] After the autopsy, hands should be washed with 0.5% chlorine solution before taking off the gloves. Furthermore if gloves have to been taken off in any condition, hands should be washed for every time. In any instances of injuries, wound should be washed with soap, mouth or nose should be rinsed with fresh water, eyes should be rinsed with either fresh water or isotonic saline solution. Caustic solutions should not be used for cleaning injury sites. After applying antiseptic solutions like iodofrom, it should wait for two minutes before washing. Protective clothes should not be taken out of the autopsy room. Waste should be classified as general, medical or hazardous. 0.5% chlorine solution can be used for disinfection of the autopsy room.[39]

N-99 masks filters 99.9% of particles.[21,41] In our country, normal surgical masks have been used while performing autopsies. Surgical masks can filter particles equal or bigger than 0.5 μm diameter and these masks can only filter the aerosols. Although using N-99 masks have side effects like headache or acne, in our country because of being one of the high-risk countries in aspect of TB, this kind of protective masks should be used routinely during autopsy.[31,37,38]

In addition to all of these, regular health screening for autopsy workers needs to be done. Although CDC and Occupational Safety & Health Administration (OSHA) give very high priority to regular screening for tuberculosis infection in health care workers, establishing surveillance programs including whole staff of the hospital is nearly impossible. Instead of this, application of surveillance programs to the staff with high risk of TB is more preferable.

As a result; we found that TST results were statistically significantly different in physicians who performed autopsies when compared to physicians who had never performed an autopsy. A positive TST result does not often indicate an active disease and it only supports the diagnosis. Negative test result does not exclude presence of active disease. For this reason, test results should be evaluated together with clinical and radiological findings.

Our study shows that autopsy workers have a high risk for TB exposure. We suggest that autopsy workers should be examined for occupational TB exposure. Furthermore, morgue buildings and autopsy rooms of our country should be designed in accordance with the international standards, and measures should be taken in this regard without delay.

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