DURABILITY OF ANTIBODY LEVEL AFTER RABIES IMMUNIZATION AND COMPARISON OF GENE EXPRESSION WITH UNVACCINATED SUBJECTS

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Abstract. Immunization before and after exposure remain the most effective way to protect against rabies. A cross-sectional comparison of antibody level durability induced by a full (4-dose) or a 3-dose rabies vaccination was investigated and whether changes occurred in *ERK1, ERK2, MEK1*, and *CXCL10* gene expression post-vaccination. Blood samples of adult subjects who received the full (n = 32) and 3-dose (n = 19) rabies vaccine at Bakırkoy Dr. Sadi Konuk Training and Research Hospital, Department of Infectious Diseases and Clinical Microbiology, University of Health Sciences Turkey, Istanbul, Turkey were taken 6-18 months post-vaccination for assay of antibody and expression levels of the above-mentioned genes. Antibody levels in about 10 % of subjects receiving both vaccination regimen decreased below protective level (>0.5 IU/ml) within 12 months post-vaccination, but there were no differences in expression levels of the four test genes in the two vaccinated groups compared to unvaccinated individuals (n = 30). In conclusion, subjects receiving both rabies vaccination regimens should be given a booster shot within one year to maintain an adequate prophylactic immune status.

Keywords: antibody level, gene expression, immunization schedule, rabies, Turkey, vaccine

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INTRODUCTION

Rabies virus leads to progressive encephalitis resulting in high mortality unless medical intervention is administered after exposure to possible rabid animals (Johnson *et al*, 2010; Lankester *et al*, 2014). More than 29 million people annually are exposed to animals already or suspected of being infected with *Rabies lyssavirus*, resulting in considerable financial burden from expenses incurred from of pre- (PrEP) and post-exposure prophylaxis (PEP) (Hampson *et al*, 2015). The most effective protection of subjects at risk of exposure to rabies is by administering PrEP, and as the incubation period of rabies in humans ranges on average from 15 to 90 days, clinical progression of rabies after exposure can be effectively prevented/treated by PEP (Tarantola *et al*, 2019). Rabies still causes death due to neglect of possible exposure and/or from insufficient vaccination regimen despite availability of effective vaccines for humans and animals (Taylor and Nel, 2015).

In Turkey, purified inactivated rabies vaccines produced in Vero cells are used, with a recommended PEP regimen of administration of rabies vaccine on day 0, 3, 7, and 14 post-suspected exposure for a subject not previously vaccinated (Rupprecht *et al*, 2010; RTMH, 2021). A fifth dose on day 28 is recommended in immunocompromised patients. For a previously vaccinated subject, two booster doses of rabies vaccines are recommended on day 0 and 3. A 3-dose regimen is administered if the animal responsible is alive. Administration of Human Rabies Immune Globulin (RIG) ensures neutralization of virus entry into axons prior to stimulation of adequate immunity by the rabies vaccine (Rupprecht *et al*, 2010). In our country, rabies immunoglobulin obtained from horse blood, which has similar clinical properties to those obtained from humans, is used in the first exposure and in the presence of high risk (WHO, 2018). However, the mechanism and duration of RIG or usefulness of a tetanus vaccine administered together with PEP and their effects on anti-rabies antibody production are not known as measurement of antibody levels is not a routine practice.

An antibody level of 0.5 IU/ml obtained by a serum neutralization test is considered an indication of adequate vaccination in people at risk of exposure to rabies (Moore and Hanlon, 2010). Because vaccine-induced immunity often persists for years, booster would be recommended only if rabies-virus neutralizing antibody titers fall to <0.5 IU/ml (WHO Publication, 2010).

Mitogen-activated protein kinases (MAPKs) (*eg* CXCL10, ERK1, ERK2, and MEK1) are involved in several pathways transducing extracellular signals to intracellular responses, such cell division, cell viability, cell differentiation and apoptosis (Gui *et al*, 2017). Many viruses manipulate host cell ERK-MAPK pathway for optimal viral replication (Manjunatha *et al*, 2017), but the role of

Rabies lyssavirus in this process remains unknown.

Here, a cross-sectional study was conducted on antibody levels 6-18 months following vaccination of subjects who received full (4)- and 3-dose rabies vaccination regimens as a result of exposure to suspected *Rabies lyssavirus*-infected animals and to compare CXCL10, ERK1, ERK2, and MEK1 gene expression profiles between vaccinated and unvaccinated individuals. The findings should assist in determining the length of immune protection and the need and timing of a booster vaccination.

MATERIALS AND METHODS

Participants recruitment

Participants were recruited from individuals who received rabies vaccination at the Sultangazi Lutfiye Nuri Burat State Hospital, Republic of Turkey Ministry of Health, Istanbul, Turkey in 2018. Participants were divided into two groups, namely, those who received a full (4)- and a 3-dose regimen of 0.5 mL of 2.5 IU/kg body weight Wistar Rabies PM/WI 38-1503-3M strain rabies vaccine (Abhayrab®; Human Biologicals Institute, Udhagamandalam, Tamil Nadu, India) was administered *via* intramuscular route into the deltoid muscle. The vaccination procedure was carried out in accordance with the recommendations of the Rabies Prophylaxis Guideline of Republic of Turkey Ministry of Health (RTMH, 2019). Passive immunization was achieved by implementing rabies immune globulin (RIG) of a single dose of 40 IU/kg equine rabies immunoglobulin (Equirab®, Bharat Serums and Vaccines Ltd, Mumbai/ Maharashtra, India) around the bite wound. Patients' information was retrieved from vaccination records of the Hospital and was reviewed by two different researchers. Individuals who received two doses or lower, booster or repeated doses of vaccines were excluded from the study.

Patients who received their last vaccination 6 to 18 months ago were invited to Bakırkoy Dr. Sadi Konuk Training and Research Hospital, University of Health Sciences Turkey, Istanbul, Turkey via phone call in order to obtain serum samples to determine antibody levels.

The study protocol was approved by the Ethics Committee of Bakırköy Dr. Sadi Konuk Training and Research Hospital (no. 2018/437). Prior written consent was obtained from all participants.

Laboratory protocols

Serum was prepared from blood sample (5 ml) by letting stand at ambient temperature for 10-20 minutes and centrifuging at 3000 rpm for 20 minutes. Serum samples were stored at -80°C until used. Levels of anti-rabies virus antibody was measured using a Sandwich-ELISA method (Anti-RV ELISA Kit; Andy Gene Biotechnology,

Co Ltd, Beijing, PR China) in replicate. For determination of gene expression, RNA was isolated from whole blood sample (200 µl) using a MasterPure™ Complete DNA and RNA Purification Kit (Lucigen Corporation, Middleton, WI) and purity ($A_{260\ nm}/A_{280\ nm}$ ≥2.0) and concentration determined using a Denovix DS-11 spectrophotometer (DeNovix Inc, Wilmington, DE). RNA (250 ng) was converted to cDNA using a Roche Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany) and RT-qPCR was performed using Syber Green SYBR Green Master Mix of Roche (Roche Diagnostics, Mannheim, Germany) in a Roche LightCycler® 480 System (Roche Diagnostics, Mannheim, Germany) with *GAPDH* as internal control (Barber *et al*, 2005). The primer sequences are listed in Table 1.

Statistical analysis

Qualitative variables are reported as frequency and percentage and quantitative variables as arithmetic mean and standard deviation (SD). Chi-square test was employed for comparisons between two categorical variables and independent samples t-test for comparisons between categorical and quantitative variables. A p-value <0.05 is considered statistically significant. Calculations were carried out using a SPSS 25 package (IBM, Armonk, NY).

RESULTS

In 2018, 2627 individuals, 1098 (41.8%) females and 1529 (58.2%) males applied to the Lutfiye Nuri Burat State Hospital (Republic of Turkey Ministry of Health, Istanbul, Turkey) vaccination center and 2581 of them were vaccinated.

The implementation preferences of the vaccines were 4-dose and 3-dose for 1263 (49%) and 453 (17.5%) subjects, respectively. Dogs and cats were responsible for 1033 (39.3%) and 1514 (57.6%) of the bites, respectively. No wild animal was reported. Equine Rabies

Table 1

Primers used in the study

Gene Name	Forward	Reverse
ERK 1	TGGCAAGCACTACCTGGATCAG	GCAGAGACTGTAGGTAGTTTCGG
ERK2	ACACCAACCTCTCGTACATCGG	TGGCAGTAGGTCTGGTGCTCAA
MEK1	GATGAGCAGGAGCGAAAGCG	CTCCCTTATGATCTGGTTCC
CXCL10	GAACTGTACGCTGTACCTGCA	TTGATGGCCTTCGATTCTGGA
GAPDH	GCATCTTCTTTTGCGTCG	TGTAAACCATGTAGTTGAGGT

Immunoglobulin (ERIG) was implemented around the bite wound in 32 (1.2%) participants. Tetanus vaccine was administered to 1356 (51.6%) subjects that were considered to be non-immunized.

Fifty-one participants (age (mean ± SD) = 46 ± 28 years) who were persuaded to return and gave blood samples 6-18 months after the last dose of rabies vaccine were included.

Thirty-two participants (average age = 48 years, ranged 24-71 years) who had received the full 4-dose vaccine regimen consisted of 21 (66%) males and 11 (34%) females, among whom 15 (47%) were injured by cats and 17 (53%) by dogs, with injuries sustained to head and face (n = 1, 3%), lower (n = 8, 25%) and upper (n = 2, 6%) limbs, but sites of the majority (n = 21, 66%) were not reported (Table 2).

Nineteen participants (average age = 42 years, ranged 16-67 years) who had received the 3-dose vaccine regimen consisted of 13 (68%) males and 6 (32%) females, among whom 10 (53%) were injured by cats and 9 (47%) by dogs, with injuries sustained to lower (n = 1, 5%) and upper (n = 3, 15%) limbs, but most sites of injuries (n = 15, 80%) were unreported (Table 2). RIG injection (rabies immunoglobulin) (n = 34, 67%) were performed at the wound sites and booster tetanus vaccine injection (n = 31, 61%) also were administered.

Among participants receiving the 4-dose rabies vaccination regimen, 8/9, 1/12 and 2/11 had anti-rabies antibody level above the protective level (>0.5 IU/ml) after 6, 12 and 18 months, respectively, ie after 6, 12 and 18 months 89, 8 and 18% respectively remained protective against rabies infection (Table 2). Among participants receiving the 3-dose rabies vaccination regimen, 5/6, 1/8 and 0/5 had anti-rabies antibody level above the protective level after 6, 12 and 18 months respectively, *ie* after 6, 12 and 18 months 83, 12 and 0% remained protective against rabies infection (Table 2).

No statistically significant changes were observed in expression of *CXCL10*, *ERK1*, *ERK2*, and *MEK1* in blood samples of rabies vaccinated participant compared to control subjects (n = 30) (Fig 1).

DISCUSSION

Post-exposure rabies vaccination regimens are carried out by administering a 4-dose vaccine, while a 3-dose regimen is performed if the animal responsible is alive. Our study reveals adequate antibody response was above 80% within 6 months post-vaccination but declined to less than 10% by 12 months of application of either the 4- and 3-dose regimen. Previous studies of PrEP by a 3-dose vaccine in young volunteers ranging from 6-43 years of age reported adequate antibody levels within 12 months post-vaccination (Xu *et al*, 2021). Older individuals develop a lower antibody response (Mastroeni *et al*, 1994, Xu *et al*, 2021). However, there

Table 2

Information regarding participants receiving full (4)- and 3-dose rabies vaccination regimen, Bakırkoy Dr. Sadi Konuk Training and Research Hospital, University of Health Sciences Turkey, Istanbul, Turkey (2018)

Participants receiving 4-dose vaccination regimen[a]													
Characteristics				Information regarding wound			Information regarding animal of concern				Rabies immuno-globulin injection	Booster tetanus vaccine injection	Anti-rabies antibody level[b] (IU/ml)
ID	Gender	Age (Year)	Time elapsed after vaccination (months)	Site	Feature	Treatment	Animal type	Status of animal	Owner status	Vaccination status			
1	M	24	6	NR	Superficial	Yes	Dog	Suspected	SA	Unvaccinated	No	No	0.38
2	M	50	6	NR	Superficial	Yes	Cat	Healthy	OA	Vaccinated	No	Yes	0.85
3	M	52	6	NR	Superficial	Yes	Cat	Healthy	SA	Vaccinated	No	Yes	0.72
4	F	58	6	NR	Superficial	No	Dog	Healthy	SA	Unvaccinated	No	Yes	0.53
5	M	28	6	NR	Deep	No	Cat	Suspected	SA	Unvaccinated	No	Yes	0.78
6	M	46	6	NR	Deep	Yes	Dog	Suspected	SA	Unvaccinated	No	No	1.44
7	F	56	6	NR	Superficial	Yes	Cat	Suspected	SA	Unvaccinated	No	Yes	0.61
8	M	51	6	NR	Superficial	Yes	Cat	Suspected	SA	Unvaccinated	No	Yes	0.60
9	M	54	6	NR	Deep	No	Cat	Healthy	OA	Unvaccinated	No	Yes	1.62
10	F	33	8	NR	Superficial	Yes	Cat	Suspected	SA	Unvaccinated	No	Yes	0.19
11	F	36	8	NR	Superficial	Yes	Dog	UO	SA	Unvaccinated	No	Yes	0.16
12	M	71	8	NR	Superficial	Yes	Dog	UO	SA	Unvaccinated	No	Yes	0.14

Table 2 (cont)

Participants receiving 4-dose vaccination regimen[a]													
Characteristics				Information regarding wound			Information regarding animal of concern				Rabies immuno-globulin injection	Booster tetanus vaccine injection	Anti-rabies antibody level[b] (IU/ml)
ID	Gender	Age (Year)	Time elapsed after vaccination (months)	Site	Feature	Treatment	Animal type	Status of animal	Owner status	Vaccination status			
13	M	55	11	NR	Superficial	Yes	Dog	Suspected	SA	Unvaccinated	No	No	1.06
14	M	53	11	NR	Superficial	Yes	Cat	Suspected	SA	Unvaccinated	No	Yes	0.15
15	F	47	11	NR	Superficial	Yes	Dog	UO	OA	Unvaccinated	No	No	0.14
16	M	47	11	NR	Superficial	Yes	Cat	UO	SA	Unvaccinated	No	Yes	0.15
17	M	62	12	NR	Superficial	Yes	Dog	UO	SA	Unvaccinated	No	Yes	0.15
18	M	44	12	NR	Superficial	Yes	Cat	Suspected	SA	Unvaccinated	No	Yes	0.11
19	F	32	12	NR	Deep	Yes	Dog	Suspected	SA	Unvaccinated	Yes	Yes	0.13
20	M	31	12	NR	Superficial	No	Cat	UO	SA	Unvaccinated	No	Yes	0.12
21	F	53	12	HN	Superficial	No	Cat	UO	OA	Unvaccinated	No	No	0.13
22	F	55	13	LL	Superficial	Yes	Dog	Suspected	OA	Unvaccinated	No	Yes	0.16
23	M	62	14	NR	Superficial	Yes	Dog	Suspected	SA	Unvaccinated	No	No	0.14
24	M	46	14	HF	Superficial	Yes	Cat	Suspected	SA	Unvaccinated	No	No	0.21
25	M	79	16	LL	Superficial	Yes	Dog	UO	SA	Unvaccinated	Yes	Yes	0.13

Table 2 (cont)

Participants receiving 4-dose vaccination regimen[a]													
Characteristics				Information regarding wound			Information regarding animal of concern				Rabies immuno-globulin injection	Booster tetanus vaccine injection	Anti-rabies antibody level[b] (IU/ml)
ID	Gender	Age (Year)	Time elapsed after vaccination (months)	Site	Feature	Treatment	Animal type	Status of animal	Owner status	Vaccination status			
26	M	60	16	LL	Superficial	Yes	Dog	Suspected	SA	Unvaccinated	No	No	0.13
27	M	48	16	LL	Superficial	No	Dog	Suspected	SA	Unvaccinated	No	No	0.16
28	M	44	16	HN	Superficial	Yes	Cat	Suspected	SA	Unvaccinated	No	No	0.15
29	M	26	18	LL	Superficial	Yes	Dog	Suspected	SA	Unvaccinated	No	Yes	0.57
30	F	63	18	LL	Deep	No	Dog	Suspected	SA	Unvaccinated	No	No	0.15
31	F	47	18	LL	Deep	Yes	Dog	Suspected	SA	Unvaccinated	No	Yes	0.18
32	F	44	18	LL	Deep	Yes	Cat	UO	SA	Unvaccinated	No	No	1.17

Table 2 (cont)

Participants receiving 3-dose vaccination regimen[a]													
Characteristics				Information regarding wound			Information regarding animal of concern				Rabies immuno-globulin injection	Booster tetanus vaccine injection	Anti-rabies antibody level[b] (IU/ml)
ID	Gender	Age (Year)	Time elapsed after vaccination (months)	Site	Feature	Treatment	Animal type	Status of animal	Owner status	Vaccination status			
1	F	21	6	NR	Deep	No	Cat	Suspected	SA	Unvaccinated	No	No	0.71
2	M	64	6	NR	Deep	No	Dog	Healthy	OA	Vaccinated	No	Yes	0.46
3	M	67	6	NR	Superficial	No	Cat	Suspected	SA	Unvaccinated	No	Yes	0.62
4	F	29	6	NR	Deep	No	Cat	Suspected	SA	Unvaccinated	No	Yes	0.99
5	M	42	6	NR	Deep	No	Dog	Healthy	SA	Unvaccinated	No	No	0.65
6	F	49	6	NR	Deep	Yes	Cat	Suspected	SA	Unvaccinated	No	Yes	0.61
7	M	36	7	UL	Superficial	No	Cat	UO	SA	Unvaccinated	No	Yes	0.17
8	M	18	7	NR	Superficial	No	Cat	Suspected	SA	Unvaccinated	No	No	0.17
9	M	16	7	LL	Superficial	Yes	Dog	UO	OA	Unvaccinated	Yes	No	0.26
10	M	30	8	NR	Superficial	Yes	Cat	Suspected	SA	Unvaccinated	No	Yes	0.16
11	F	59	12	HN	Superficial	Yes	Cat	Suspected	SA	Unvaccinated	No	No	0.19
12	M	65	12	NR	Superficial	No	Dog	UO	OA	Unvaccinated	No	No	0.15
13	F	47	12	NR	Superficial	Yes	Cat	Suspected	SA	Unvaccinated	No	No	2.98

Table 2 (cont)

Participants receiving 3-dose vaccination regimen[a]													
Characteristics				Information regarding wound			Information regarding animal of concern				Rabies immuno-globulin injection	Booster tetanus vaccine injection	Anti-rabies antibody level[b] (IU/ml)
ID	Gender	Age (Year)	Time elapsed after vaccination (months)	Site	Feature	Treatment	Animal type	Status of animal	Owner status	Vaccination status			
14	M	29	12	NR	Superficial	Yes	Dog	Suspected	SA	Unvaccinated	No	No	0.21
15	M	48	13	NR	Superficial	Yes	Dog	UO	OA	Vaccinated	No	No	0.19
16	M	38	13	NR	Superficial	No	Dog	Suspected	SA	Unvaccinated	No	No	0.12
17	M	54	14	NR	Superficial	No	Dog	UO	OA	Unvaccinated	No	No	0.16
18	F	47	14	NR	Superficial	Yes	Cat	UO	SA	Unvaccinated	No	No	0.15
19	M	40	18	UL	Superficial	Yes	Dog	UO	SA	Unvaccinated	Yes	No	0.14

[a]0.5 ml of 2.5 IU/kg body weight Wistar Rabies PM/WI 38-1503-3Mstrain rabies vaccine (Abhayrab®; Human Biologicals Institute, Udhagamandalam, Tamil Nadu, India)

[b]6-18 months post-last vaccine dose; adequate level >0.50 IU/ml (Anti-RV ELISA Kit Andy Gene Biotechnology Co Ltd, Beijing, PR China)

ID: identification number; M: male; F: female; LL: lower limb; NR: no report; HF: head/face; HN: hand; UL: upper limb; UO: under observation; OA: owned animal; SA: stray animal

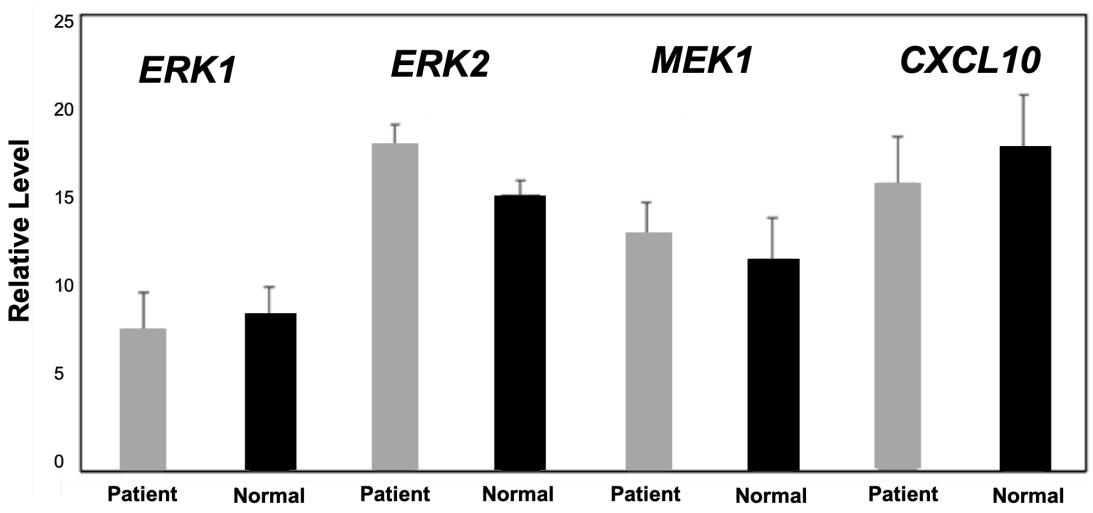

Fig 1 - Expression levels of *CXCL10, ERK1, ERK2,* and *MEK1* of 30 normal subjects and 51 patients 6-18 months subsequent to rabies vaccination, Bakırkoy Dr. Sadi Konuk Training and Research Hospital, University of Health Sciences Turkey, Istanbul, Turkey (2018)

Gene expression level in whole blood was measured by RT-qPCR relative to internal *GAPDH* control. Vertical line indicates standard deviation.

was a marked lack of patient records on wound category and depth, but the low frequency of RIG implementation would suggest wounds were mostly superficial. The 4-dose vaccination schedule vaccination regimen was implemented in 49% of the victims. The completion rate of rabies vaccination ranges 17-92% depending on age, gender, community, and country (Mazigo *et al*, 2010; Tenzin *et al*, 2011; Esmaeilzadeh *et al*, 2017; Tran *et al*, 2019).

Completion of a rabies vaccination regimen after exposure to a potentially rabid animal is crucial, but does guarantee complete protection (Scrimgeour and Mehta, 2001). Both human Rabies Immunoglobulin (hRIG) and equine Rabies Immunoglobulin (eRIG) are considered to have similar clinical efficacy and either one is recommended by WHO (2018). RIG is more preferred if bitten by a dog, monkey or bat (Soentjens *et al*, 2021). However, patients who receive RIG treatment often feel a sense of safety and therefore are less likely to complete the full vaccination course (Tran *et al*, 2019). In addition, RIG treatment may influence antibody response and increasing

the dose of RIG may reduce the immunogenicity of the vaccine (Warrell, 2014). Treatment with monoclonal antibodies may be an alternative to RIG adjuvant therapy against rabies and such a product was approved in India (Sparrow *et al*, 2019).

Antibody response against vaccination may be influenced by some factors. Besides serological parameters, B and T cellular response are also important in rabies immunity (Overduin *et al*, 2019). Furthermore, when antibody is detected in serum, it rarely occurs in the cerebrospinal fluid and may have limited penetration into the CNS, where it is most needed (Johnson *et al*, 2010).

However, these studies were carried out in healthy subjects, and undetected co-morbidity and other underlying factors entities affecting antibody response in healthy subjects may have been overlooked. Obesity negatively impacts on antibody production by influencing the immune system (Sirikun *et al*, 2018). In addition, subcutaneous administration of vaccines may lead to low immune response (Yamamoto *et al*, 2019) as well as concurrent administration of rabies and tetanus-diphtheria (Td) vaccines (Gozdas *et al*, 2018)

Previous studies reported levels of protective antibody levels range 70-90%, even in cases with complete vaccination regimens (Kaya Kilic *et al*, 2016) indicating administration of booster vaccination will be needed within one year for subjects at risk of repeated exposure. No time point was recommended as the longest time interval after the initial vaccination schedule to increase vaccination response (Strady *et al*, 2009; Wieten *et al*, 2013). Langedijk *et al* (2018) stated administration of a booster dose within the first year after intradermal or intramuscular vaccination produces sufficient antibody response that is more permanent. Rapid anamnestic response is generated years after implementation of PreP or PEP in clinical studies as memory cells produced following primary vaccination have a long lifespan (Tarantola *et al*, 2018). Another important advantage of PrEP compared to subjects who received a full-dose PEP schedule is that in the former situation specific anti-rabies antibodies produce higher and faster anamnestic reaction with higher affinity following implementation of a booster dose (Khawplod *et al*, 2007).

At present, the number of studies evaluating rabies vaccination efficacy and adequacy of immune response is progressively increasing (Yamamoto *et al*, 2019; Soentjens *et al*, 2021; Mills *et al*, 2021). A recent study demonstrated MAPK pathway is activated and expression of proinflammatory chemokines increases following rabies virus infection (Liu *et al*, 2020). Expression of CXCL10 and CCL5 in microglia is regulated by activation of multiple signaling pathways mediated by recognition of rabies virus infection (Nakamichi *et al*, 2005).

MEK1/2-ERK pathway mediates the expression of CXCL10 in murine macrophages in response to rabies virus infection (Nakamichi *et al*, 2004). However, there is no study addressing how these gene levels change after anti-rabies vaccination. We show expression of *CXCL10, ERK1, ERK2,* and *MEK1* are not significantly different between vaccinated and normal control subjects. The genes have similar expression levels between two populations. These findings imply that CXCL10, ERK1, ERK2 and MEK1 might be less dosage tolerant.

Observational studies carried out on anti-rabies vaccination are more reliable and ethical than randomized-controlled studies for evaluation of anti-rabies vaccine and changes in PEP regimens. Limitations of our study are (i) employment of an ELISA technique for detection of antibody level whereas the gold standard is the rabies rapid fluorescent focus inhibition test (RFFIT) (Moore and Hanlon, 2010), (ii) compilation of data from only a single-center and (iii) small number of volunteer rabies vaccinated participants limiting statistical analysis among parameters that may impact the efficacy and duration of the two vaccination regimens.

In conclusion, our study highlights the importance of early intervention and implementation of booster vaccine dose within one year after the last vaccination if post-exposure prophylaxis is to be effective.

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CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflicts of interest.

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