REFINEMENT OF SALIVA MI-RNA BIOMARKERS FOR SPORT-RELATED CONCUSSION

REFINAMENTO DE BIOMARCADORES DE SALIVA MI-RNA PARA CONCUSSÃO RELACIONADA AO ESPORTE

REFINAMIENTO DE LOS BIOMARCADORES DE MI-ARN DE LA SALIVA PARA LA CONMOCIÓN ENCEFÁLICA RELACIONADA CON EL DEPORTE

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Cuifeng Gu[1]
(Physical Education Professional)
Guojian He[1]
(Physical Education Professional)
Chenhong Lin[2]
(Physical Education Professional)

1. Hebei University of Economics and Business, Physical Education Department, Hebei, Heibei, China.
2. Hebei Sport University, Department of Human Sports Science, Hebei, Heibei, China.

Correspondence:
Guojian He
Hebei, Heibei, China.
gjianhe@163.com

ABSTRACT

Introduction: The changes in brain structure caused by a sports-related concussion may initially be indistinguishable, however, the irreversible deleterious effects are noted in the long term. An early diagnosis may provide the patient with a better recovery chance and increased survival. For this purpose, this paper studies the feasibility of a diagnosis for concussion by microRNA (mi-RNA) biomarkers contained in the saliva of athletes. Objective: Verify whether salivary miRNAs could be considered good biomarkers for sports concussion. Methodology: Salivary mi-RNA levels were determined from 120 saliva samples of 120 players. There were 43 with a diagnosis of concussion and 77 without a diagnosis of concussion. Samples from players with a concussion were collected 30 minutes prior to activity, samples from individuals who did not engage in physical activity were also compared. Results: On the evaluation of 30 miRNA from individuals with a concussion between contact and non-contact sports there was high detection reliability(P<.05). Both miR-532-5p and miR-182-5p showed reduced amounts of physical activity. The miRNA-532-5p and miRNA-182-5p show significant results among 43 subjects from pre-exercise to post-exercise. The miRNA-4510 showed a significant result (p < 0.05) between contact and non-contact sport types. The amount of miRNA-4510 expanded in 20 individuals in the contact sport at post-exercise but remained normal in the non-contact sports group. Conclusion: The salivary miRNAs are reliable biomarkers for concussion. ***Evidence Level II; Therapeutic Studies – Investigating the results.***

Keywords: Saliva; MicroRNAs; Biomarkers; Brain Concussion; Athletic Injuries.

RESUMO

Introdução: As alterações da estrutura cerebral provocadas por uma concussão relacionada ao esporte podem ser inicialmente indistinguíveis, porém os efeitos deletérios irreversíveis são notados a longo prazo. Um diagnóstico precoce poderá fornecer ao paciente uma chance maior de recuperação e aumento de sobrevida. Para tanto, estuda-se a viabilidade de um diagnóstico de concussão por biomarcadores de micro RNA (mi-RNA) contidos na saliva de esportistas. Objetivo: verificar se os miRNAs salivares são biomarcadores confiáveis para concussão esportiva. Metodologia: Os níveis de mi-RNA salivares foram determinados a partir de 120 amostras de saliva de 120 jogadores. Haviam 43 com diagnóstico de concussão e 77 sem diagnóstico de concussão. Amostras de jogadores com concussão foram coletadas 30 minutos antes da atividade, amostras de indivíduos que não praticaram atividade física também foram comparadas. Resultados: Na avaliação de 30 mi-RNA de indivíduos com concussão entre esportes de contato e sem contato houve grande confiabilidade de detecção(P<,05). Tanto o miR-532-5p quanto o miR-182-5p mostraram quantidades reduzidas na atividade física. O miRNA-532-5p e o miRNA-182-5p mostram resultados significativos entre 43 indivíduos desde o pré-exercício até o pós-exercício. O miRNA-4510 mostrou um resultado significativo (p < 0,05) entre os tipos de esporte com contato e sem contato. A quantidade de mi-RNA-4510 expandiu-se em 20 pessoas no esporte com contato no pós-exercício, mas permaneceu normal no grupo de esporte sem contato. Conclusão: Conclui-se que os miRNAs salivares são biomarcadores confiáveis para concussão. **Nível de evidência II; Estudos Terapêuticos - Investigação de Resultados.**

***Descritores*:** *Saliva; MicroRNAs; biomarcadores; Concussão encefálica; Traumatismos em Atletas.*

RESUMEN

Introducción: Los cambios en la estructura cerebral causados por una conmoción encefálica relacionada con el deporte pueden ser inicialmente indistintos, sin embargo, los efectos deletéreos irreversibles se manifiestan a largo plazo. Un diagnóstico anticipado puede proporcionar al paciente una mayor posibilidad de recuperación y una mayor supervivencia. Por lo tanto, se estudia la viabilidad de un diagnóstico de conmoción encefálica mediante los biomarcadores de microARN (mi-ARN) contenidos en la saliva de los deportistas. Objetivo: verificar si los miARN salivales son biomarcadores fiables para la conmoción encefálica deportiva. Metodología: Se determinaron los niveles de mi-ARN salival a partir de 120 muestras de saliva de 120 jugadores. Había 43 con diagnóstico de conmoción encefálica y 77 sin diagnóstico de conmoción encefálica. Las muestras de los jugadores con conmoción encefálica se recogieron 30 minutos antes de la actividad, también se compararon las muestras de los individuos que no practicaban actividad física. Resultados: En la evaluación de 30 miRNA de individuos con conmoción encefálica entre deportes de contacto

y sin contacto hubo una alta fiabilidad de detección (P<.05). Tanto miR-532-5p como miR-182-5p mostraron cantidades reducidas en la actividad física. El miRNA-532-5p y el miRNA-182-5p muestran resultados significativos entre los 43 sujetos desde antes del ejercicio hasta después del mismo. El miRNA-4510 mostró un resultado significativo (p < 0,05) entre los tipos de deporte de contacto y de no contacto. La cantidad de miARN-4510 se expandió en 20 individuos en el deporte de contacto en el momento posterior al ejercicio, pero se mantuvo normal en el grupo de deporte sin contacto. Conclusión: Llegamos a la conclusión de que los miARN salivales son biomarcadores fiables para la conmoción encefálica. ***Nivel de evidencia II; Estudios terapéuticos - Investigación de resultados.***

Descriptores*: Saliva; MicroARNs; Biomarcadores; Conmoción Encefálica; Traumatismos en Atletas.*

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INTRODUCTION

Almost eight million students of United States who participate in high school athletics and more than four hundred eighty thousand students who compete as Athletic Association (NCAA) athletes are at risk for concussion and subconcussive injuries.[1,2] A lot of studies have proved that both clinically diagnosed concussion and subconcussive traumas initiate indistinguishable changes in brain structure and functions.[3,4] These changes comprise variations in white matter and cerebrovascular integrity, blood flow, brain activation during working memory tasks, resting state functional connectivity, and brain chemistry as measured by various forms of magnetic resonance imaging (MRI).[3,5,6] Subconcussive impacts can cause deleterious effects on brain function and neuro degeneration in selected individuals.[7,8]

Concussion is a clinical diagnosis established upon history of injury, neurological examination, neuropsychological testing, and, sometimes on neuroimaging. Clinical evaluation of concussion in athletes can be conducted by measurement of brain specific biomarkers with the help of a blood test. These results can probably lead the way towards management decisions.[9–13] Studies are being carried out to evaluate use of biomarkers in providing diagnostic and prognostic information.[13] A novel set of biomarkers, called microRNAs (mi-RNA), are considered as next generation of biomarkers for study of cancer, cardiovascular and neurodegenerative diseases.[14] MiRNAs are 17–29 nucleotides endogenous RNA molecules capable of controlling post-transcriptional level protein synthesis. MiRNAs can be ascertained in serum and can act as an index of disease pathology. Cerebrospinal fluid, serum, and urine have abundant MiRNAs. These MiRNAs are stable at variable pH conditions, and are unaffected by repeated freeze thaw and enzymatic degradation. These properties make MiRNAs superior over protein-based markers.

The usefulness of MiRNAs as diagnostic markers of mild TBI has been investigated by our group and others.[15–18] In 2016, Bhomia and co-workers identified specific and sensitive miRNA-based biomarkers for mild and moderate TBI using real time polymerase chain reaction (PCR) methodology.[16] A comparative study was conducted between samples of mild to severe TBI subjects with trauma and normal control patients and 10 miRNA signatures were recognised. Eight of these miRNAs showed significantly increased expression in those subjects with bruises on computed tomography (CT). Moreover, Johnson and co-workers detected a group of miRNAs in saliva that were associated with prolonged post-concussive manifestations at 4 weeks post-injury.[18]

This study assessed the performance of a group of 12 MiRNAs biomarkers (miR-27a-5p, miR-1246, miR-30e-3p, miR-30a-3p, miR-151a-3p, miR-30e-5p, miR-1307-5p, miR-182-5p, miR-3074-5p, miR-629-5p, miR-944 and miR-27b-3p), and their correlation with severity of injury after mild to severe TBI.[16]

Methodology

After taking the approval of the protocol review committee and institutional ethics committee a study was carried out. Total 50 saliva mi-RNAs with diagnostic power for SRC[19] by removing mi-RNAs surprise by chronic or acute exercise. 310 saliva samples from 200 sports persons, with ages of 10- 55 years were included in this study.

Inclusion criteria

- Active sports player
- Concussion within 24 hrs

Exclusion criteria

- Pregnant women players
- Any dental disease
- Any neurologic problem
- Alcoholic player
- Players with respiratory infection

Impact of acute exercise

To evaluate the acute role of exercise on concussion-related mi-RNAs and estimate whether exercise can cause changes in saliva mi-RNAs. Results vary between sports persons in non-contact or contact persons. We determined the mi-RNA amount from 120 saliva samples from 60 sports players. All the samples were collected from players before starting sports activity, and again within 20 minute of exercise fulfilment. In noncontact sports, 50 persons were included.

Role of chronic exercise

The amount of mi-RNA from 200 saliva samples of 100 sports players was determined. All the samples were collected from the players of hockey, basketball, soccer or mixed- martial arts training. After completion of sports activity again saliva samples were collected for determination.

Diagnosis of SRC

We determined salivary mi-RNA levels from 120 saliva samples collected from 120 sports players. 43 persons with concussion and 77 persons without concussion were studied. Concussion samples of players were collected before 30 minute of sports activity and other samples were collected from individuals who did not participate in sports activity. 20 people were involved with sports activity and 23 were non-sport related persons.

Sports played with non SRCs were included in manner to evaluate whether a predictive serotype using saliva mi-RNAs would carry out likewise for non SRCs and SRCs.

Basic characteristic parameters like age, gender and comorbidity were studied. Presence or absence of old concussion was self-explained by the person. Saliva was collected from each person following oral water rinse, in a non-fasting state, using swabs. All the samples were stored at normal temp for up to 58 days. Samples were then incubated at 48˚C and frozen at 21˚C prior to RNA analysis.

Saliva RNA analysis

From the mi-RN easy tool kit RNA was isolated from every sample. The quality of RNA was evaluated by the analyzer. Each mi-RNA parameter was scaled. Down-stream evaluation concentrated on 30 mi-RNA people last studies of traumatic brain injury.[19, 20]

Statistical analysis

Perfection of every one mi-RNA was evaluated by area under the AUC. Specificity, Sensitivity, + ve /-ve likelihood ratios and differences in groups were also determined for every mi-RNA.

RESULTS

Table 1 shows that general parameters of players like depression (5.33%), anxiety (4.67%), hyperactivity disorder (4.67%), etc. 26.67% of people had already suffered from concussion. (Figures 1, 2 and 3)

Three mi-RNAs had effect of sports individuals. miRNA-532- 5p and miRNA-182-5p show significant result among 43 sports person pre-exercise to post-exercise. miRNA- 4510 with a significant result ($p < 0.05$) between exercise sport type. mi-RNA-4510 amount expands in 20 contact persons after post exercise but remains normal in non-contact persons. We found 8 miRNAs that expand and 12 mi-RNAs that reduce. In acute exercise miRNA-532-5p had shown reduced amounts. Amount of miRNA-29c-3p grow head impacts (R =.62, p =.007), amount of miRNA-26b-3p reduce with head impacts (R = .55, p = .019) (Table 2).

The 12/30 salivary mi-RNAs unchanged by acute exercise were detection of SRC. 9 out of 12 show a significant difference ($p < 0.05$) between non-concussed and concussed person, and 3 miRNAs show average results (AUC ≥ 0.70). The mi-RNA for transforming non-concussed and concussed persons was miR-27a-5p (sensitivity = 82%, AUC = 0.84, specificity = 83%). miR-27a-5p and miR-30a-3p show a relation and get the maximum perfection (sensitivity = 84%, AUC = 0.825, specificity = 75%) for differentiating concussion status.

DISCUSSION

This is a novel study to examine levels of serum MiRNAs in subjects who were symptompless but suffered multiple, elevated subconcussive effects during the course of a sports season. This prospective study introduces a group of 12 miRNA biomarkers (miR-27a-5p, miR-1246, miR-30e-3p, miR-30a-3p, miR-151a-3p, miR-30e-5p, miR-1307-5p, miR-182-5p, miR-3074-5p, miR-629-5p, miR-944 and miR-27b-3p). This study estimated the performance of a group of MiRNAs biomarkers using not only standard methods of concussion evaluation, but also advanced assessment technology.

This work identified salivary MiRNAs that are likely to detect SRC without the erratic effects of acute exercise or season-long training. With an AUC of 0.825, a single MiRNA ratio detected concussion status. This precision is comparable to that of serum biomarkers. 21-28 It is difficult to establish the effect of acute or chronic exercise on SRC biochemical markers. 29-31. The new study highlights crucial links between saliva MiRNAs.

Most clinicians who undertake side-line evaluations of athletes rely heavily on balance tests, cognition testing, and symptom reports to detect SRC. 32 Only when baseline assessments are available can these systems perform flawlessly. As a result, sportsmen who want to avoid suspension might use these tactics.[21] Only qualified experts must be permitted to give Neurocognitive testing,[22] and the time necessary for

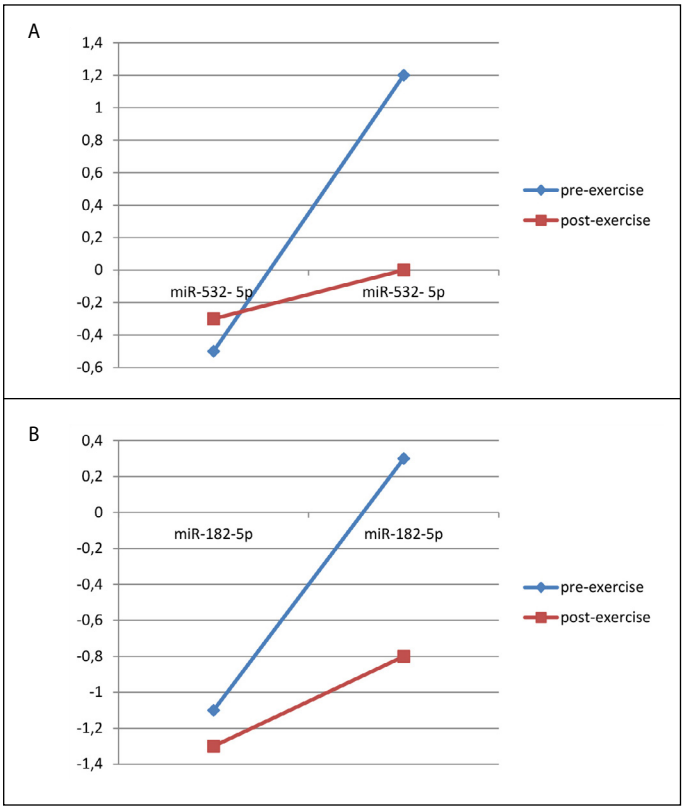

Figure 1. A and B show that evaluation of 30 concussion mi-RNA among contact or noncontact with an effect of exercise (P<.05). miR-532-5p and miR-182-5p both show reduced amounts with exercise.

Table 1. Basic parameter of participants.

	Acute exercise	Sports season	Total	SRC	Non-SRC	Exercise control	Control
Basic parameter	100	50	120	20	23	31	46
Gender	74	29	70	12	16	21	29
Age	24±12	21±17	19±25	22±14	23±21	21±32	23±74
Height	67±21	69±23	66±27	69±69	69±11	70±26	71±32
Weight	167±13	168±19	165±74	170±26	171±22	169±19	170±69
BMI	24±74	25±25	23±63	26±12	25±36	26±12	26±11
Depression	8	0	6	NA	5	1	NA
Anxiety	4	3	1	NA	1	NA	NA
Head injury details							
Past concussion	28	12	35	7	4	11	13
Hits to head	4	-	-	-	-	-	-
TBI collection (h)	-	-	-	12±61	14±51	-	-
Oral							
Time of collection (h)	9am	10am	11am	1pm	2pm	11am	10am
Dietary restrictions	6	5	5	10	2	1	3

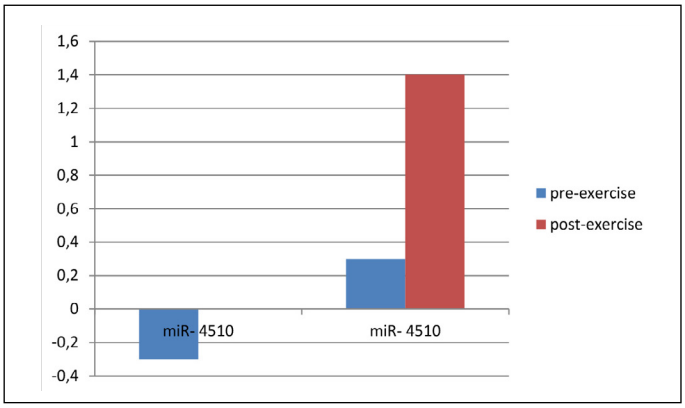

Figure 2. miR-4510 in contact sports people show expanded results.

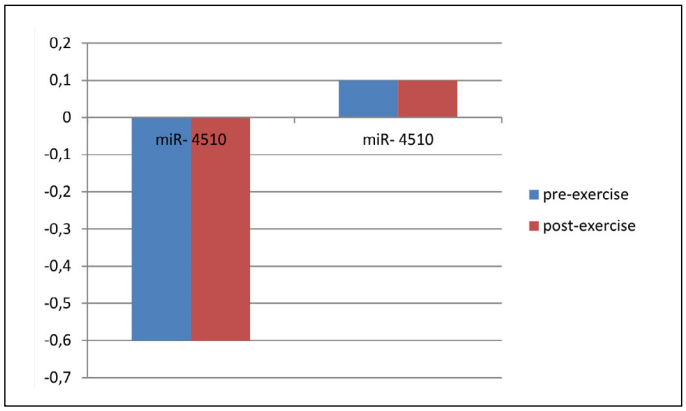

Figure 3. No correlation is found between miRNA-532-5p head impact and among contact sports person (P = 0.42, R = 0.18), miRNA-182-5p (R = 0.17, P = 0.53,), or miR-4510 (P = 0.39, R = 0.21).

Table 2. 12 salivary mi-RNAs unchanged by acute exercise.

	AUC	Sensitivity	Specificity	+ve LR	-ve LR
miR-27a-5p	0.84	0.82	0.83	2.81	0.41
miR-1246	0.83	0.71	0.79	3.82	0.56
miR-30e-3p	0.79	0.81	0.71	2.42	0.41
miR-30a-3p	0.78	0.81	0.72	2.12	0.42
miR-151a-3p	0.77	0.80	0.70	2.09	0.39
miR-30e-5p	0.73	0.58	0.68	2.19	0.62
miR-1307-5p	0.73	0.69	0.61	1.74	0.51
miR-182-5p	0.65	0.61	0.53	1.68	0.59
miR-3074-5p	0.65	0.51	0.68	1.59	0.81
miR-629-5p	0.58	0.06	0.91	0.81	1.11
miR-944	0.55	0.69	0.42	1.22	0.69
miR-27b-3p	0.53	0.59	0.39	1.11	0.89

testing precludes side-line administration.[23]The creation of an objective saliva MiRNA evaluation for concussion is a critical step in overcoming these limitations. Rapid results may be obtained using salivary MiRNAs with only a minimum medical knowledge. Previous research has found that miR-27a and miR-30a levels in blood or saliva can be used to diagnose concussion. [24]The use of serum markers necessitates the pricking and removal of blood, which might result in infection. Athletic trainers can readily measure sensitive and specific salivary MiRNAs, resulting in faster and more accurate examinations. These tests are crucial for assessing athletes who have brief symptoms and pass side-line testing but are suspected of having an underlying brain damage. Both miR-27a and miR-30a are expressed by peripheral neurons and play key roles in pain modulation and cerebral ischemia-reperfusion damage.[25,26] SRC examination results in subjective symptoms and functional abnormalities caused by brain damage, but these are unable to identify brain injury itself. The incorporation of biomarkers miR-27a and miR-30a into SRC pathophysiology may aid in the accurate identification of brain damage. For example, in the current investigation, two molecules implicated in concussion pathophysiology (miR-26b-3p and miR-29c-3p) were shown to change near the end of a contact sports season and to correlate with the occurrence of non-concussive head impacts. Validation of this association in a larger, longer-term cohort of contact sport athletes using sensitive measures of head impact stress might eventually lead to the development of a biological test for non-concussive head impacts that increase SRC risk.

The goal of this extensive study is to look at how saliva MiRNA changes with acute exercise and cumulative sports activity. The time between activity and saliva collection varies depending on the subject. Because previous research has demonstrated that exercise can influence miRNA levels for up to 24 hours, subjects with SRC were included within 24 hours after injury.

Our work intends to find saliva MiRNAs that alter between sports, so that they may be used as useful SRC sideline indicators. Participation in contact sports causes alterations in saliva miRNAs. The main elements that influence these MiRNAs include cumulative exhaustion, musculoskeletal injury, or conditioning.

CONCLUSION

We concluded that salivary miRNAs are effective concussion biomarkers. We investigate a group of salivary mi-RNAs that are affected by acute activity in contact sports. Salivary mi-RNAs that are unaffected by exercise serve as an excellent biomarker participant for SRC.

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